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CYTO-GENETICS AND THE SPECIES-CONCEPT[1]

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ONE'S concept of species must necessarily involve one's point of view concerning fundamental questions such as the following: Do species really exist? If so how do they originate? And how may species be recognized?

For the sake of brevity I shall assume that my audience accepts the evidence from the ever-increasing body of experience in classifying animals and plants, which certainly indicates that species do exist and that they are really natural groups of individual organisms. That is to say, with organisms in which large numbers of individuals have been thoroughly studied, the experience of many students of classification goes to show that really distinct groups of individuals have existed and do exist; that while the individuals within a group are not all alike and may differ almost endlessly and even strikingly in certain details, yet they resemble one another more than they resemble the individuals of any other group; also that the groups existing in a given time-plane usually differ from one another in numerous characteristics and are separated by definite gaps, marked by physiological and ecological divergence if not by constant morphological features, except in some cases where natural hybrids occur. Along with a certain range of variation to

[1] Read at the Fifth International Botanical Congress, Cambridge, England, August 21, 1930.

be found among the individuals composing these natural groups, there exists a relative constancy or stability for each group taken as a whole. Relative stability, combined with a definite tendency to vary, seems to be generally characteristic of these natural groups. It is this combination of the idea of the existence of natural "unit-groups" of organisms, now generally referred to as Linneons, and the idea of perpetuation of these groups over long periods of time which I am assuming to be acceptable to most biologists as a basis for the formulation of a species-concept.

Thinking of species in these terms, we may consider briefly the second question, How do species originate? My purpose in taking up this question here is not to discuss evolutionary processes in detail, but merely to take the next logical step in formulating a concept of species which shall be consistent with existing knowledge. The perpetuation of these specific natural groups is made possible by the reproduction of individuals. Sexual reproduction requires the union of two unlike gametes, and in the higher organisms these unlike gametes come from different individuals, except in self-fertilized organisms and forms which reproduce asexually or by some modified form of sexual reproduction. It is pertinent to this discussion, therefore, to inquire as to what extent the individuals of different specific groups intercross naturally and what bearing this may have on our concept of species. Many cases of intergrading forms have been found to connect up distinct groups which, except for these connecting forms, would rank as species. In some such cases experiments have proved that the connecting forms are really hybrids,[2] and in many others the evidence from the intergrades themselves is fairly conclusive. It is now generally accepted that many true species will hybridize and that they often produce partially fertile offspring. In plants especially hundreds of interspecific hybrids have been produced by artificial

[2] See especially the recent research of Marsden-Jones and Turrill on *Silene*, Kew Bull. Miscel. Infor. 1928–1929.

crossing and all degrees of fertility have been found in the first generation plants from fully fertile to fully sterile. The general tendency, however, is toward sterility and at the same time it must be remembered that a great number of such crosses have been attempted and have failed completely. If along with this evidence we consider the evidence that species, once established, tend to remain relatively stable or fairly constant over considerable periods of time, we are led to infer that, while the individuals within species may intercross freely and be highly interfertile, the individuals of different species hybridize comparatively rarely in nature and when they do cross the hybrids are more or less sterile. It must be noted in passing, however, that very recent experiments on artificial plant hybrids have proved that fully fertile, constant hybrids (amphidiploids and autosyndetic hybrids such as *Crepis artificialis*) may sometimes be produced by crossing two very distinct species. Furthermore, there is steadily accumulating evidence that in certain plant genera such as *Hieracium, Rosa* and *Betula,* interspecific hybridization is rather common and sometimes gives rise to very numerous hybrid forms which perpetuate themselves by some form of apomixis. Many such forms have been described as species and their hybrid nature has been discovered later by cyto-genetic investigations. Natural hybridizing of species, therefore, may be recognized as one method of origin of species without invalidating our concept of species. But from existing evidence it is clear that interspecific hybridization can hardly be thought of as the only process making evolution possible.

Further evidence bearing on this question of how species originate has come from intensive studies of plant and animal groups which have led to the publication of monographic treatises on classification. Such studies have shown the existence within species of sub-specific groups, often occupying different geographic areas and differing more from one another in morphology or interfertility or both than do the individuals

within the subgroups. The very existence of such subspecies or varieties is generally looked upon as good evidence that Darwin was correct in his fundamental idea that the existing differences between many species are the result of gradual accumulation of such smaller differences as distinguish the subgroups within a species. At this point it is unnecessary to discuss the genetic processes which make possible this differentiation within species. These will be mentioned when considering the bearing of the evidence from cyto-genetics on the species-concept.

Existing evidence seems to indicate that both the Darwinian and the Lotsyan hypotheses of species origin are correct in part; *i.e.*, many of our present species must have arisen through gradual differentiation, aided by isolation, within preexisting species and, on the other hand, some of our present species are probably the result of natural hybridization followed by the establishment of one or more new constant forms combining some of the characteristics of the parent species. Apparently there is nothing in either of these hypotheses as to origin of species which is inconsistent with our concept of species as thus far developed. The essential ideas in this concept may be summarized as follows:

(1) Common structural characteristics which unite certain individual organisms into one group, the species.

(2) Characteristic features which distinguish such groups from one another.

(3) Relative stability combined with more or less variability within the group.

(4) Common descent of all the individuals of the group from one or more preexisting species.

(5) Free intercrossing and high (but not necessarily complete) interfertility among the individuals of the group.

(6) Absence of free intercrossing and usually low fertility if not complete sterility in hybrids between different species (although highly fertile and constant new forms may sometimes arise in this way).

(7) The frequent occurrence of subspecific groups, often occupying different geographic areas which differ more from one another in structure or interfertility or both than do the individuals composing each subgroup.

The third question raised as pertinent to our problem of formulating a species-concept was, How may species be recognized?

The recognition of types and the preparation of diagnoses or detailed descriptions of types were essential methods of the museum or herbarium worker who frequently recognized as "species" whatever new individuals failed to correspond sufficiently in his judgment to any species already recognized. Many of the so-called species which were named and classified by this method turned out later to be merely forms, varieties or subspecies of the same or some other species. When the partial failure of older methods became more apparent other aids were invoked. Some writers emphasized *distinctness* as a valuable criterion, but well-marked varieties or subspecies are very distinct. Others, notably Darwin, emphasized *constancy* or the ability to breed true. This is of much greater value, especially if used in the general sense of constancy in the more important distinguishing characters of the species. We might combine the two and speak of constant distinctness between true species. But within most species there is variation from generation to generation and never strict constancy.

The growing dissatisfaction with former methods of classification led to various suggestions and proposals, some of them being so radical as to advocate abolition of the term species from biology. Another radical proposal was that of Lotsy who advocated gametic purity as the sole test of the validity of species. But if this criterion were adopted the number of species would become inconceivably large and the natural unit-groups, the Linneons of Lotsy, would still exist and require classification. Other scholars, notably Poulton, appealed to taxonomists to name new species with reservations and with a deter-

mination to seek for further evidence which might or might not warrant their permanent recognition.

More recent trends in taxonomic work have placed emphasis on field study, including geographic distribution and ecological relations, the use of statistical methods in describing species, the development of methods of experimental ecology and finally the application of the results of investigations in genetics and cytology to problems of classification. Each of these developments contributed something to the species-concept. Supplementing museum work with field study brought to light many significant facts concerning the geographic areas occupied by related species, with resulting inferences as to their comparative ages and phylogenetic relations, and this in turn had a bearing on the principle of common descent of the individuals within a species. The use of statistical methods in the study of variation within a species has been of value in establishing the existence of subspecies in many plants. The work on experimental ecology has thrown considerable light on the nature of the variations found among the individuals of which species are composed. Thus Turesson, in Sweden, has shown that many species of plants which are wide-spread and occupy different habitats are composed of different hereditary races which he calls ecotypes because they are distinct forms which through selection have become adapted to different sets of ecological conditions. This is further evidence of such differentiation within species as may lead eventually to the origin of new species. Again, Hall, in California, by his transplant experiments has been able to confirm Turesson's findings and has also shown that certain supposed "species" are merely transient forms induced by peculiar environmental conditions. These modified forms do not retain their original distinguishing features when transplanted to a common testing garden under uniform conditions, which shows that their features are temporary variations induced by the environment. This in turn necessitates the revision of our descriptions of the particular species in question.

In brief we find that all these more refined methods of study have yielded results which strengthen our conviction as to the validity of the seven basic ideas with which we have attempted to formulate a concept of species.

The contributions of genetics and cytology to the species problem will be considered somewhat more in detail. A concept of species, to be consistent with the facts of evolution, must harmonize the apparently paradoxical phenomena of constancy of species and variety of forms within species. The integration of these diverse tendencies is adequately provided for in the known material basis of heredity and genetic variation.

I. The Basis of Heredity

There is a distinctive, relatively stable, hereditary complex for each species. This relatively stable heritage consists of many different primary elements, the genes, which are carried in a practically constant group of chromosomes, peculiar to the species. This constant chromosome group consists of n pairs of chromosomes where $n =$ the number of chromosomes in the gametes of the species. Each chromosome carries a linear series of genes and in each pair of chromosomes the genes are homologous and normally occupy identical loci. With reference to the nature of the genes they carry, each pair of chromosomes differs from every other pair but the genic differences between the chromosomes are not necessarily accompanied by visible differences in the chromosomes of a species. In many genera of plants the chromosomes are so small or so similar in size and shape that no constant morphological difference between them can be observed. In many such genera, however, number of chromosomes has proved a very useful criterion, an outstanding example being J. Clausen's work on the genus *Viola*. On the other hand, in some groups of organisms the number, size and shape of the chromosomes are very useful as additional criteria for the recognition of species and the determination of relationship between species. These criteria, however, should

always be used in connection with all other available criteria. In accepting the chromosomes as the chief material basis of heredity, one should not overlook the cytoplasm and the important rôle it plays in development of the individual; and certain actually demonstrated cases of cytoplasmic inheritance must be admitted. But, in view of available evidence, the chromosomes must be recognized as the basis of Mendelian heredity, and it appears that heredity is mostly Mendelian in all plants above the thallophytes if not in them.

II. The Genetics of Variation

The differences found among the individuals within species have been rather extensively analyzed by cytologic and genetic methods. In the wild state the individuals in a population of any sexually reproduced animal or plant exhibit both structural and functional differences from one another. Many of these variations are merely the somatic effects of environmental differences and are not inherited, but some of these differences among the individuals in wild populations are found to be inherited. Furthermore the study of various populations of the same species, especially populations from different geographic regions, reveals the existence of still more inherited differences. During the decade just closing a vast amount of work has been done in analyzing the genetics of these intraspecific hereditary differences. As a result of such investigation we are now in position to generalize to some extent.

Intraspecific differences which are inherited fall into several different categories according to the nature of the germinal changes involved. The most important of these categories, considered with reference to the origin of species, are three in number as follows:

1. *Genic variation:* Genic differences in an otherwise normal chromosome complex condition both qualitative and quantitative differences among the individuals of a species. In a single species of fruit fly hundreds of such varietal differences are known and as a rule each pair of

differences involves only one pair of genes. Similar simple genic differences are known in hundreds of other animals and plants. Also a great many varietal differences are known, especially quantitative differences, which involve two or more pairs of genes simultaneously. These genic differences arise *de novo* as gene mutations which can be produced experimentally. The existence of these numerous genic differences makes possible the occurrence of innumerable natural subspecific forms resulting from various combinations of genes in cross-fertilized species, and in self-fertilized species natural populations are more or less heterogeneous due to gene mutations and occasional crossing. That these gene mutations function as the basic process in the origin of species has been advanced by Morgan, Muller and others. The existence of highly polymorphic species in which the numerous subspecific forms differ in respect to individual genes but which exhibit a uniform chromosome complex certainly indicates that gene mutations make possible intraspecific differentiation. But genic variations, whether due to chemical changes in individual genes or occasional loss or addition of genes, are inadequate to account for the differences in chromosome number and morphology which exist among the species of many genera to say nothing of similar differences between genera, and between the larger natural groups.

2. *Chromosomal variation:* Rearrangements of parts of chromosomes have been found in nature. A fragment of a chromosome has been added to the normal chromosome complex in certain species of *Crepis, Nicotiana* and other genera. In maize a chromosome of one variety may be represented by two smaller chromosomes in another variety. Sometimes such a fragment becomes attached to one of the other chromosomes. Such rearrangements of parts of chromosomes sometimes affect intraspecific variation; they also seem the most likely cause of known cases of differences in arrangement of corresponding genes in closely related species of Drosophila. In this connection it should be noted that

chromosomal variations including both translocation and deletion of parts of chromosomes can be caused experimentally; also that studies of chromosomes in relation to phylogeny in several plant genera, notably in *Muscari,* in some of the *Polygonaceae,* in *Vicia* and more recently in *Crepis* have produced considerable evidence that the evolution of these genera has been accompanied by gradual transformation in chromosome size, and has led to the conception that changes in chromosome number may have arisen in this way. On the other hand, the well-known deleterious effects on the organism of gross unbalance in the chromosome complex makes it necessary that such hypothetical transformation of the chromosomes proceed very gradually, by minute subtractions or additions in an order of magnitude corresponding to very small groups of genes, or individual genes or even parts of genes. Whether such gradual transformation of chromosomes is actually going on in nature and whether this process is more or less fundamental than gene mutation in the general method of evolution remains to be discovered.

3. *Polyploidy,* or balanced increase of the entire chromosome complex, sometimes produces very distinct forms of existing species. The most commonly occurring polyploid forms are triploids and tetraploids. Triploids are only slightly fertile, yet even with low fertility they may sometimes serve as the starting point in the origin of a series of polyploid forms as has been shown by M. Navashin in his work on triploids in *Crepis capillaris.*[3] Tetraploids are self-fertile and usually more vigorous than the diploid ancestor. Furthermore they frequently do not cross readily with the diploid form and when they do the progeny are mostly triploids which are sterile. All this favors perpetuation of tetraploid forms. Thus in polyploidy we find a process which makes possible the fairly rapid rise of a new species with $4n$ chromosomes from a $2n$ progenitor. In view of existing

[3] M. Navashin, "Studies on Polyploidy I. Cytological Investigations on Triploidy in *Crepis,*" Univ. Calif. Publ. Agr. Sci. 2: 377–400. 1929.

evidence, however, polyploidy, like interspecific hybridization, in general seems to be of secondary importance in the origin of species. The primary processes appear to be gene mutation and chromosome transformation. These ideas are not in conflict, necessarily, with Lotsy's suggestion that hybridization may have been of importance in the origin of certain phyla.

Polyploidy and other variations in chromosome number have made necessary some modification of the old idea that each species has just one specific number of chromosomes. Thus in all the true polyploid forms derived from the same diploid species the same specific chromosome complex occurs throughout, although intraspecific variation may be considerably increased. And if, morphologically, such a group of forms may still be considered one species, then the mere fact of such variation in chromosome number need not prevent such taxonomic treatment. Again, a few cases of variable chromosome numbers within species are known, for example, *Viola canina*[4] and *Crepis syriaca*.[5] Morphologically each of these groups must be considered as one species. They must be looked upon as special cases or exceptions to the general rule that all the members of a species have the same chromosome number.

We may now return to the species-concept previously formulated and amplify each of the seven essential ideas in the light of evidence from cytology and genetics. These may then be stated somewhat as follows:

1. Common structural characteristics which unite certain individual organisms into one group, and a common genetic basis for the group represented by a specific chromosome complex or *genom*.

2. Characteristic features which distinguish such groups from one another, one of these features frequently being the chromosome garniture (*genom*).

[4] J. Clausen, unpublished data.

[5] L. Hollingshead and E. B. Babcock, "Chromosomes and Phylogeny in *Crepis*," Univ. Cal. Publ. Agr. Sci. 6: 1–53. 1930.

3. Relative stability combined with more or less variability within the group. This stability is made possible by a high degree of regularity in chromosome distribution from cell to cell and from parent to offspring, while inherited variations arise from occasional changes in genes and chromosomes.

4. Common descent of all individuals of the group from one or more preexisting species is made possible by the known mechanism of heredity and genetic variation.

5. Syngamy or free intercrossing and high interfertility among the individuals of the group are just what would be expected in organisms in which a large majority of the genes in all the chromosomes are homologous.

6. Absence of free intercrossing and usually low fertility if not complete sterility in hybrids between different species (with a few exceptions including amphidiploids) are logical results of the accumulation of genic and chromosomal differences between diverging groups of individuals within a species.

7. The existence of subspecific groups often occupying different geographic areas, which differ more from one another in structure and interfertility or both than do the individuals composing each subgroup, but these subspecific groups sometimes still connected with one another by intergrading forms. This is the necessary result of genetic variability within the species plus the influence of environmental variability, isolation and natural selection. It is a stage in one process of species formation.

Thus it appears that the evidence from cyto-genetic research on intraspecific and interspecific variation can be combined with the basic ideas in a species-concept so as to add considerably to the definiteness of the concept. Furthermore, cytology has brought forth additional useful criteria for distinguishing species, especially the number and morphology of the chromosomes. And finally cyto-genetic investigations are coming into general use for testing the genetic nature of plants suspected of being hybrids and as a helpful method of determining the degree of relationship between species of the same genus.

It will be noted that nowhere in this paper has a *definition* of the term species been proposed. Darwin considered the term to be indefinable and yet it is obvious that he made continual use of a very definite concept of species. It has long seemed to the writer that it should be worth while to attempt to formulate such a concept in the light of present-day knowledge and it is hoped that the foregoing propositions will prove useful as a temporary working basis; no doubt these ideas will need to be extended or modified. Furthermore, it should be noted that the phylogenetic concept of species has not been considered here inasmuch as this aspect of the problem has been discussed in earlier publications.[6, 7]

In this connection should be noted the excellent and timely contribution of Vavilov, represented among the abstracts of papers to be read at the Fifth International Botanical Congress. Although this paper was not delivered, the abstract presumably will appear in the published proceedings of the congress under the title: "The Linnaean species as a system." That Vavilov is dealing with essentially the same concept of species, and in the same relation to evolution, as the present writer, is indicated by the following paragraphs quoted from his abstract.

> Thus, *a Linnaean species is,* according to our conception, *a separate morpho-physiological system connected in its genesis with a definite environment and area.*
>
> In opposition to the rather common notion as to the relativeness of species and the conventional character of their understanding, the great number of concrete facts encountered in studying the cultivated plants and their wild relatives, has induced us to recognize the Linnaean species as actual complexes, actual systems, which exist in nature and represent important definite links in the evolutional chain, the knowledge of which is very helpful in mastering the multifariousness of the organized world.

[6] H. M. Hall and F. C. Clements, "The Phylogenetic Method in Taxonomy," Carnegie Institution Publ. 326: 3–30. 1923.

[7] L. Hollingshead and E. B. Babcock, "Chromosomes and Phylogeny in *Crepis*," Univ. Cal. Publ. Agr. Sci. 6: 1–53. 1930.

The concrete material shows that the so-called "Linnaean species" may be very different in regard to their contents. Being more or less separate systems, they manifest themselves in a *different compass.* The analysis of a great number of Linnaean species by means of the method of differential systematics and differential geography, as well as by the modern methods of genetics and cytology, reveals the great diversity of the species. Thus, there are sometimes bulky systems belonging to one Linnaean species, which might be expediently subdivided into categories or subspecies. Sometimes, on the contrary, a Linnaean species represents a very limited and comparatively small system.

The conception of a species must not be obliterated. Through our knowledge of the multifariousness shown by cultivated plants and by their relatives, of plant groups where the chaos of diversity seems to be boundless, we have come to conceive of a species as a regular system which may embrace categories of different compass. The species, as a conception, is necessary not only for the sake of convenience, but for a real comprehension of the essence of the evolution process. The process of evolution may proceed uninterrupted on its fundamental lines, but it has nodes in its chain, which are the species representing systems of hereditary forms.

In conclusion let me emphasize the importance of using all possible criteria for recognition of species in pursuing the work of classification, especially in preparation of taxonomic monographs. General works on floras and faunas are indispensable and in these classification will doubtless continue to be based primarily and largely on morphological resemblances and differences together with data on geographic distribution. But in the detailed study of restricted groups of organisms as many as possible of the essential features of the species-concept should be tested. This requires cytologic and genetic study which in turn calls for living material; it also calls for field observations including ecological relations, barriers, species boundaries, overlapping and occurrence of hybrid forms. Although difficult, time consuming and expensive, such comprehensive research offers a really worth-while opportunity for a broad and intensive attack on fundamental biological problems.

CYTOLOGICAL EVIDENCE AS TO THE STATUS OF DROSOPHILA MELANOGASTER

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In 1925 the author published mainly in collaboration with Professor G. C. Hicks, now of the University of Buffalo, a number of articles dealing with the cytological conditions present in the reduction divisions of *D. melanogaster*, which in the United States and to a large degree in the world beyond is the *corpus vile* of genetical teaching. These were three in number and appeared in *Science*, the AMERICAN NATURALIST and in *Genetica*. The conclusions stated in these various articles were that the cytology of this interesting and most variable species clearly showed it to be of heterozygous or hybrid origin, since its reduction divisions presented an exact parallel to those found in interspecific and intergeneric hybrids. The reactions of geneticists in this country and abroad have had in the interval time to develop. The general attitude of American genetical workers has been either that of silence or contempt. The Europeans as is generally the case have shown themselves to be not only more open-minded but also of broader outlook than most of their American colleagues. It is unfortunately assumed by many American geneticists that the cytology of *D. melanogaster* is of little importance compared with its genetical behavior. This situation is all the more remarkable because American geneticists in general hold much more confidently to the chromosome theory of inheritance than do their European colleagues. Frequently, too, those who are favorably disposed towards cytological investigations assume that the results obtained in the author's laboratory are erroneous or the result of the use of inferior technique. This attitude is very frequently present in personal discussions but is

more rarely manifested in publication, where naturally more prudence in statement must be observed.

The first important criticism of our work on *Drosophila melanogaster* is that published in 1928 by Belar in Lieferung 5 of the "Handbuch der Vererbungswissenschaft" edited by Baur and Hartman (Berlin, Gebrueder Borntraeger, 1928). The author in this case supports his criticisms by photomicrographs, which are supposed to show a normal behavior of the chromosomes in the reduction divisions. All the photomicrographs of the divisions in the primary spermatocyte indicate a clear lagging of chromosomes, precisely as first described by us in 1925. The illustration of the second division, however, does show a normal behavior of the chromosomes, but that is frequently found to be the case in known hybrids as well as in numerous extremely variable species of plants, which are with good reason suspected to be of hybrid origin. Belar attacks our technical procedure, suggesting that our faulty technique has caused us to confuse mitochondria (chondriosomata) with chromosomes. A very frequent criticism of our work in fact has been directed at our supposed confusion of chromosomes with other structures of the cell, not only mitochondria but also even nucleoli or plasmosomata. These criticisms will be dealt with later in connection with our own observations.

In an important communication published in an English résumé in the seventh volume of the publications of the Genetical Bureau of the University of Leningrad, Zuitin asserts the chromosomal character of the lagging bodies present particularly in the first reduction mitoses of *D. melanogaster* and in this respect corroborates the description published by us in 1925. He further emphasizes the abnormal character of the reduction division of *D. melanogaster* and interprets it as an indication of the hybrid interspecific origin of this species.

More recently Miss League has published a summary of her observations on the meiotic phenomena of this

much discussed species[1] in which she concedes the abnormal character of the reduction divisions in the species but interprets the lagging structures not as chromosomes but as plasmosomata.

Later the present author discussed and criticized Miss League's observations[2] as well as a less important communication by Huettner.[3] It was pointed out that it was impossible to regard the lagging bodies which are particularly clearly present in the first reduction division as plasmosomata. Huettner in his article cites an article published recently in *La Cellule* in opposition to our views but has no evidence based on his own observations of the male mitoses to offer. He particularly commends, however, the use of Feulgen's reaction for the detection of chromatin as destined to settle the argument in favor of the point of view of American Drosophilists. The use of Feulgen's reagent, however, has not proved favorable to the view of the critics of the chromosomal character of the lagging bodies in the reduction divisions of *D. melanogaster,* for these structures unmistakably give the reactions of chromatin and consequently can not be regarded as related either to chondriosomata or plasmosomata. These important observations published by Woskressensky and Scheremetjewa[4] seem to set at rest the chromosomal nature of the lagging bodies of which such fantastic explanations have been recently furnished by recent authors, apparently unfamiliar with the cytological peculiarities of hybrids. The present author has likewise treated a large amount of material of the species under discussion with Feulgen's reagent and is able to confirm absolutely the observations of the two authors just cited. Not only do these authors find the abnormal lagging bodies present in the mitoses of the reduction

[1] *Science,* 71: 99, January 24, 1930.

[2] *Science,* 71: 315–17, March 21, 1930.

[3] *Science,* 71: 241, February 28, 1930.

[4] "Die Spermiogenese bei *Drosophila melanogaster* Meig," *Zeitschrift fuer Zellforschung u. mikroskopische Anatomie,* Bd. 10, Heft 2, 15 January, 1930.

phase of the species under discussion to be chromosomal but they also note the same multiplication of number in the chromosomal equipment in the reduction division earlier described and figured by Dr. Hicks and the present author. This is a further feature of known or justly suspected interspecific hybrids. Its presence has been the special object of ridicule of purely experimental

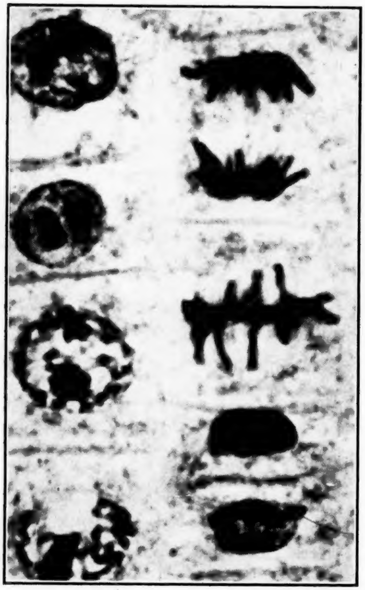

Fig. 1

biologists. It seems clear that the experimental methods alone in the biological sciences can lead to no permanently important result. The comparative and morphological data are obviously necessary to complete the picture.

It is now possible to describe our own mature observations on the meiotic phenomena of *D. melanogaster*. It will serve a useful purpose in this connection to refer first to the somatic and reproductive divisions in that

classic object of cytological investigation *Allium cepa*, the common onion. Fig. 1 is a reproduction of a highly magnified photomicrograph of the nuclear divisions of this species as seen in the tips of the roots. On the left of the figure are seen cells in stages of transition from the resting conditions to that of karyomitosis. The second cell from the top on the left shows a typical resting nucleus. In the cell above it and the two below the nucleolus is passing into the active stage and the nuclear reticulum is giving place to a definite thread-like and much convoluted spireme. In all the stages described

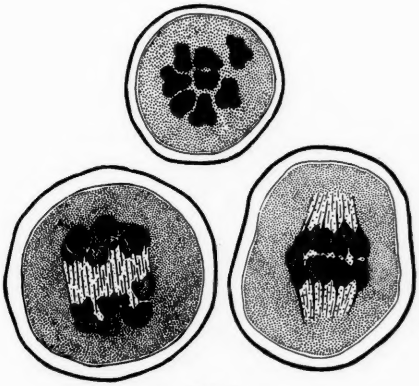

FIG. 2

and shown on the left of the photogram, in addition to the nuclear filament or spireme, there is present a darkly staining body (sometimes two or more), the nucleolus, which is sometimes surrounded by a lighter halo. To the right of the photogram are shown three well-recognized stages in nuclear division, namely, the metaphase, anaphase and telophase of mitosis. In these only chromosomes or their derivative the spireme can be distinguished. The nucleoli so distinctive of the resting stage and the earlier phases of preparation for division are conspicuous by their complete absence. In Fig. 2 are shown the reduction phenomena of *Allium cepa*. In the

item at the top of the figure the metaphase of mitosis is shown in polar view, exhibiting eight chromosomes, half the number shown in the somatic or sporophytic divisions. To the right below appears the metaphase in lateral view, showing clearly in this aspect the synaptic mates or bivalent chromosomes, which in this phase appear to be related end to end (telosynaptic mates). To the left appears the anaphase of the meiotic first division. Here again we see nothing whatever of lagging bodies of any description, whether plasmosomes or nucleoli. The illustrations, photographic and graphological, sporophytic and gametophytic, show in fact no indication of lagging bodies either in the metaphase or anaphase. This is the general condition in clearly defined species and may consequently be described as the normal condition for normal species.

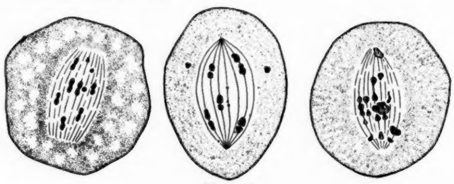

Fig. 3

We may now turn with advantage to the meiotic conditions in known hybrids. In Fig. 3 is shown on either side the metaphase of interspecific hybrids. On the left is represented the meiotic division of a hybrid hazel (Corylus) and on the right that of a hybrid violet, borrowed from the recent publications of Woodworth on the Betulaceae and of Clausen on the genus Viola. In the center appears the reduction division of a triploid *D. melanogaster* in which appear six pairs of chromosomes constituting telosynaptic mates. The close general resemblance of this figure to the meiotic divisions of the two known hybrids appearing on either side is obvious.

In Fig. 4 appear the phenomena of the anaphase of meiosis in the species under discussion. On the left is

shown the anaphase as represented in our article published in 1925. Obviously in this phase as in the preceding metaphase there is marked lagging of bodies resembling in every respect chromosomes. The next item to the right represents one of Zuitin's figures, showing a somewhat later stage of the anaphase. The lagging and extrusion of what is apparently chromosomal material is as marked here as in the illustration immediately to the left. In both figures there is a perinuclear halo of mitochondrial material, which on account of the nature of the preservative used is indistinctly seen. To the extreme right parallel figures above from the author's earlier description and below a somewhat later stage of

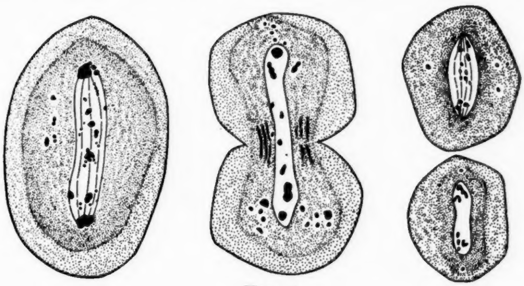

FIG. 4

the anaphase of the second division from Zuitin's article already cited. The lagging of bodies interpreted by both the present author and Zuitin as chromosomes is clearly seen.

It has been emphatically asserted by several American authors that the lagging structures represented in the two last figures are either the result of imperfect fixation or correspond to other structures of the cell than chromosomes. The accepted reagent at the present time put forward by the biochemists for the diagnosis of chromatin is that of Feulgen, which depends on hydrolysis with dilute hydrochloric acid, followed by treatment with basic Fuchsin and sulphurous acid. In Fig. 5 is shown the topographic distribution of the chromosomes in the first

meiotic division as demonstrated by this reaction. This figure is copied from the article by Woskressensky and Scheremetjewa cited above. The present author by the use of the method of Feulgen has reached substantially the same results as these authors and consequently confirms their conclusions. It has appeared better, however, to reproduce their figures rather than to present original illustrations. The two items at the top illustrate the metaphase with lagging bivalent chromosomes. The two drawings below visualize the anaphase of the first meiotic division, and furnish confirmation of the

Fig. 5

present author's observation of strongly lagging and multiplied chromosomes in this phase. Surrounding the area occupied by the spindle-shaped divisional figure are the mitochondrial or chondriosomal perinuclear zones.

The writer has been severely although not very logically criticized for employing Carnoy's alcohol-chloroform-acetic acid fluid for preservation. It is generally admitted by competent cytologists that this reagent is one of the best for the preservation of the chromosomal structures, which are the most important elements in the present discussion. It is true that this reagent does not

preserve so well the cytoplasmic structures. On the other hand, those reagents which have a favorable action on the cytoplasm are less successful in the case of the nucleus. It has been thought wise as a consequence to compare the action of the two types of reagent in photomicrographic illustration. Fig. 6 at the top shows three primary spermatocytes of *D. melanogaster* preserved with Carnoy's fluid. The figure is clearest in the cell on the left, where the chromosomes are scattered longi-

Fig. 6

tudinally over the spindle as univalents. A number of these have already reached the poles and constitute a dense mass in which the identity of the chromosomes is largely lost. The cell in the center is obliquely sectioned and does not as a consequence show clearly. On the extreme right of the upper item appears another anaphase, less clear than that on the left. Below on the right is seen the anaphase of the left side of the upper item, more highly magnified. On account of the twisted form and small size of the sex organs in *D. melanogaster* it is difficult to get axial sections of the cells. This situation together with the small size of the spermatocytes makes

clear photomicrographs difficult to obtain. On account of the difficulties just described, the metaphase of the primary spermatocyte is shown at different foci in the two items below and on the left of the figure. In the extreme left is shown one end of the nuclear figure in focus. The next item shows the other end in sharp focus. Only four of the telosynaptic mates appear with some degree of clearness. Two others present in this preparation are not shown, including the small rounded pair, so familiar in the figures of the somatic cells of *D. melanogaster*. In Fig. 3 the middle item shows this cell complete with the

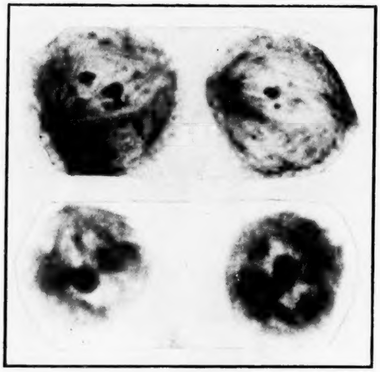

FIG. 7

six pairs of chromosomes, corresponding to the triploid condition described by Morgan in his monograph of the species, published in *Bibliographia Genetica* in 1925.

The upper item of Fig. 7 shows the secondary spermatocytes of the species in the anaphase. The lagging of the chromosomes is very obvious (preservation with Carnoy's solution). In the lower photomicrograph appear two cells in the metaphase, preserved with Champy's fluid consisting of chromic acid, bichromate of potash and osmic acid. This is one of the reagents highly prized by those whose special interest lies in the structures of the cytoplasm. To the left appears a somewhat normal

metaphase, while on the right the metaphase is characterized by the strong lagging of univalent chromosomes. It is easy to parallel such contrasting figures in the meiotic phenomena of known hybrids between species.

In Fig. 8 are shown views at different foci of secondary spermatocytes in the later stages of division preserved with Benda's fluid, consisting of chromic acid, osmic acid and a mere trace of acetic acid. The preservation of the cytoplasmic structures in this photomicrograph is particularly good. The mitochondria are arranged in two groups on either side of the equator of the dividing cell. Although the division is far advanced the chromosomes can still be seen lagging in their procession to the poles.

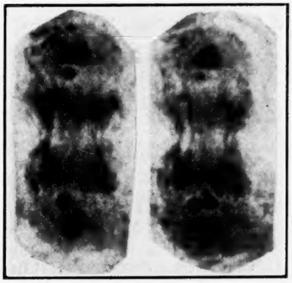

FIG. 8

It seems clear from the various data introduced in the present article that the meiotic phenomena of *D. melanogaster* are very far from normal and to any one who has a reasonably complete acquaintance with the cytology of hybrids, particularly of hybrid plants, concerning which our knowledge is less incomplete, present an unmistakable indication of hybrid contamination. The imprudent assertions made from time to time, especially in the less responsible occasions of private intercourse, as contrasted with scientific publications, as to the complete normality of this species, appear to possess no real foundation in fact. Not only are lagging bodies exactly comparable to

those present in known hybrids found in the meiotic mitoses of this species but these can be demonstrated by the best approved technique of biochemistry to be of the nature of chromatin. The strong resemblance of the maturation divisions in the male gonads in *D. melanogaster* to those of the male meiotic mitoses of known hybrids can not remain without significance to those biologists whose interests lie in the general point of view which appears to be most truly scientific. Further, the abnormalities presented by the reduction divisions of this protean species offer the best possible explanation of its extreme variability. This explanation is moreover quite in line with our ever-increasing knowledge of the cytological phenomena presented by the extremely variable species of large genera, long ago emphasized by Darwin in the "Origin of Species."

Summary

1. The use of various methods of preservation, comprising those which conserve with the greatest fidelity the nuclear and cytoplasmic structure, respectively, makes it clear that the reduction phenomena of *D. melanogaster* are quite abnormal.

2. The abnormalities present are those of known hybrids and of the often extremely variable species of large genera.

3. Assertions that the lagging structures in this species are not chromatin are disproved by the fact that they clearly give the reactions of chromatin with Feulgen's reagent.

4. Even if they were not chromatin they would constitute a thoroughly abnormal condition of which there is no reasonable explanation other than that of previous interspecific hybridization.

DO EARTHWORMS GROW BY ADDING SEGMENTS?

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Introduction

It has been a common assertion that one of the factors contributing to the growth in length of earthworms is the laying down of new segments. The process of segmentation is said to continue beyond the embryological stage of development in the cocoon. Pearl and Fuller[2] are

[1] This investigation was undertaken and completed at Ohio State University.

[2] Pearl, R., and Fuller, W. N., "Variation and Correlation in the Earthworm," *Biometrica,* 1905–06, *4,* 213–229.

Up to a certain age at least, *Lumbricus* adds somites at the posterior end of the body with growth. It is clear . . . that the great extent of the total range in the variation in this character arises from the presence of a comparatively small number of individuals with few somites which unduly extend the lower end of the range. It seems reasonably certain from what we know of the method of growth in the earthworm that if this sample of worms could have been allowed to go on growing the range of variation in total number of somites would have steadily decreased with lapse of time.

The fact that there is no apparent tendency towards extreme extension of the range at the upper end indicates that the bulk of the worms included in the sample have either completed the process of adding somites or at least that the process is going on very slowly if at all. . . .

The conclusion, that the earthworm is more variable in length than in number of somites, may seem at first sight paradoxical. But it is really no more so than to conclude, what is very obviously the fact, that man is more variable in respect to sitting height than in respect to number of vertebrae. It simply indicates that in the earthworm we have two kinds of growth occurring together: one the addition of somites, the other increase in size of the individual somites. That these two kinds of growth are acting together will be clear when the correlation surface between total number of somites and length of body is examined (p. 218).

It is seen at once by mere inspection that the relationship between the two characters is not the same in all parts of the correlation table. Thus the worms in the two lowest length classes (*i.e.,* from 10 to 15 cm in length)

even of the opinion that the addition of segments proceeds after a worm is sexually mature, although the rate of addition progressively diminishes. The place of origin of new segments is in dispute: Pearl and Fuller state that the segments are laid down at the posterior end, Morgan[3] that they are intercalated between the posterior segment and that adjoining.

The data confirming the contention that increase in length of an earthworm is partially dependent upon an increase in segments seem to have been available to these writers, although references are not cited. In so far as this conception depends for confirmation upon the statistical analysis of measurements of mature worms rather than upon the observation of changes which occur in species characteristics during developmental history one is probably justified in questioning its validity.

THE PROBLEM

The problem arose out of a chance observation of Dr. W. M. Barrows and one of the writers that the number of segments found in each of two worms (probably *Helodrilus foetidus*) just emerging from cocoons was essentially the same as that noted in mature specimens of the

are not, in general, individuals having few somites, but instead these arrays center well towards the right end of the table. The five worms having the smallest number of somites all fall in or above the third length class (p. 219).

[3] Morgan, T. H., "Regeneration," 1901, The Macmillan Co.

If we examine the method of regeneration from the posterior end of a piece of an earthworm, we find that when several or many posterior segments have been removed a new part comes back, composed at first of a very few segments. The terminal segment contains the new posterior opening of the digestive tract. New segments are now formed just in front of the terminal segment, the youngest being the one next to the end-segment. The process continues until the full complement of segments is made up. . . . Comparing these results with those described above for the anterior end, we find, in both cases, that only a few segments are at first formed, but in the posterior regeneration new segments are intercalated near the posterior end. This process of intercalation is the characteristic way in which many annelids add new segments to the posterior end, as they grow larger and longer (p. 9).

(The experiments were made upon *Allolobophora foetida*, referred to in this paper as *Helodrilus foetidus.*)

species. This observation raised the question whether the process of segmentation continues beyond the cocoon stage, or whether it terminates at some stage of embryological development prior to emergence from the cocoon.

The problem, then, is twofold:

(1) To determine whether or not the process of segmentation terminates in the cocoon stage.

(2) To obtain data showing some of the species characteristics of mature members of the species *Helodrilus foetidus* in terms of means and their probable errors, measures of dispersion and correlations between the various physical traits.

Methods

Three stages of development were selected for investigation:

(1) Worms just emerging from cocoons;

(2) Worms having no clitella (collected from the compost heap);

(3) Worms selected on the basis of clitella—these being considered indicative of sexual maturity.

Cocoons were collected from a compost heap upon the university farm in the spring of 1927, the dates ranging from April 6 to May 2. These were washed and placed upon moist filter-paper in shallow covered glass dishes. It was made a matter of routine procedure to make a daily count of the segments of the newly hatched worms and to obtain measurements of their length. This is a difficult problem because the worms are very small, and when viewed under a microscope of sufficient power to make visible all the segments, they appear almost transparent, with little indication of the notches or grooves at the septa. To remedy this the following technique was devised. The worms were removed from the dishes by means of a dissecting needle and introduced into a 2 per cent. solution of formalin. As soon as bodily movements ceased the worms were transferred to a bath of India ink and then were laid out and straightened upon a glass

slide. This was accomplished very easily before the onset of rigor which would have prevented securing an accurate measure of their length. The glass slide was then placed upon the mechanical stage of a microscope equipped with a hair line in the ocular which permitted the making of an accurate count of the segments. These were clearly differentiated by the collection of carbon particles in the intersegmental notches. Two or more segment counts were made upon each worm to insure accurate results.

The worms without clitella were selected as they were brought to light from the compost heap. It should be noted that collection of newly hatched worms in the com-

TABLE 1

	Worms	No.	Range	Mean	S. D.	P. E. of S. D.	P. E. of Mean	S. D./M(100)
Segment length in mms	A	250	.039–.242	.1208	.038	.001	.0016	31.4
	B	250	.276–.94	.5466	.120	.0036	.005	21.9
	C	250	.49–1.278	.843	.125	.0037	.005	14.8
	D	240	.49–1.278	.849	.122	.0037	.005	14.3
Body length in mms	A	250	1.5– 27	12.32	4.28	.129	.183	34.7
	B	250	29– 95	57.64	12.55	.378	.535	21.7
	C	250	49–135	88.06	13.35	.403	.57	15.1
	D	240	50–135	88.60	13.15	.405	.573	14.8
Segment number	A	250	37–136	99.65	16.95	.511	.722	17.0
	B	250	59–125	105.5	9.55	.288	.407	9.0
	C	250	67–124	105.0	9.78	.294	.417	9.3
	D	240	67–124	104.75	9.80	.301	.426	9.3

A Newly hatched worms
B Worms without clitella
C Mature worms (all)
D Mature worms (*Helodrilus foetidus*)

post is virtually impossible. Observation of the range in length of the worms (Table 1) taken discloses that the lower extreme of this group is approximately the same as the upper extreme of the newly hatched worms. The technique of killing the worms was the same as that employed in the other group, and, as before, the worms were straightened and measured before rigor occurred. In the small worms the segments were counted by means of the microscope mentioned, while those in the larger ones were counted by using a binocular microscope. The worms with clitella were killed in a stronger solution of formalin and the segments were counted by means of the binocular microscope.

Worms with clitella were collected in the spring of 1927 between April 6 and April 27; those without clitella between May 23 and June 11.

Results

In Table 1 are presented data pertaining to the length in millimeters of each segment, length of the worms in millimeters and number of segments for each selected stage of development—newly hatched, worms without clitella and mature worms. It should be noted that ten

TABLE 2

Helodrilus subrubicundus

Body length in mms	Segment number in Clitellum	Segment number ant. to Clitellum	Total segment number
79	8	21	105
78	8	21	119
67	8	21	111
72	8	21	117
49	8	21	94
83	8	21	106
81	8	21	117
79	8	21	117
79	8	21	113
92	8	21	107
Ave. 75.9	8	21	110.6

of the mature worms are of a different species (*Helodrilus subrubicundus*), the data for these being presented in Table 2 and for mature *Helodrilus foetidus* in Table 3.

TABLE 3

Helodrilus foetidus

	Range	Mean	S. D.	P. E. of S. D.	P. E. of Mean	S. D./M(100)	Correlation r	P. E. of r
Segment length	.49–1.278	0.849	0.122	.0037	.0053	14.36		
Worm length	50–135	88.60	13.15	.405	.573	14.8		
Segment number	67–124	104.75	9.80	.301	.426	9.3		
Segment number in Clitellum	4– 10	7.4	0.644	.0198	.028	8.7		
Segments anterior to Clitellum	23– 28	25.629	0.661	.020	.029	2.5		
Length with segments							+.43	±.0355
Segments in Clit. with anterior seg.							—.40	±.036
Segments in Clit. with total seg.							+.078	±.043
Segments in Clit. with length							+.145	±.0426

TABLE 4

P. E. OF DIFFERENCE OF THE MEANS FOR NUMBER OF SEGMENTS OF THE WORMS

	A			B			C		
	1	2	3	1	2	3	1	2	3
A							5.35	.834	6.42
B	5.85	.828	7.06						
C				.50	.583	.857			

A Newly hatched worms
B Worms without clitella
C Mature worms (all)
1 Difference of means
2 Probable error of difference
3 Difference of means divided by probable error of difference

It is of interest to know that a certain definite relationship observed in the number of segments anterior to and within the clitellum first gave the clue that the selection was not homogeneous with respect to species.

Table 4 gives the probable error of the difference of the means for number of segments at different stages of development. Table 5 does the same for the means of the body lengths.

TABLE 5

P. E. of Difference of Means for Body Lengths

	A			B			C		
	1	2	3	1	2	3	1	2	3
A							75.74	.257	290.8
B	45.32	.788	57.5						
C				30.42	.781	38.9			

A Newly hatched worms
B Worms without clitella
C Mature worms (all)
1 Difference of means
2 Probable error of difference
3 Difference of means divided by probable error of difference

Table 6 shows the correlation between body lengths and number of segments for each selected stage of growth.

Table 7 gives Pearl and Fuller's values for *Lumbricus herculeus* Sav.

TABLE 6

Correlation of Body Length in mms with Number of Segments

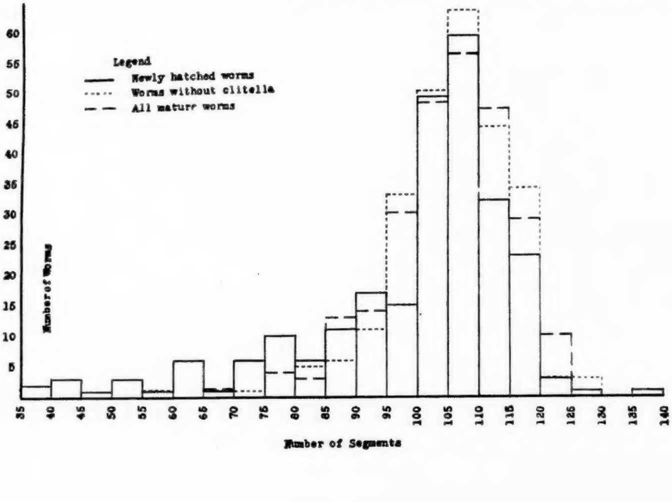
Worms	r	P. E. of r
Newly hatched worms	+ .454	± .0338
Worms without clitella	+ .239	± .040
Mature worms (all)	+ .40	± .036
Helodrilus foetidus	+ .43	± .0355

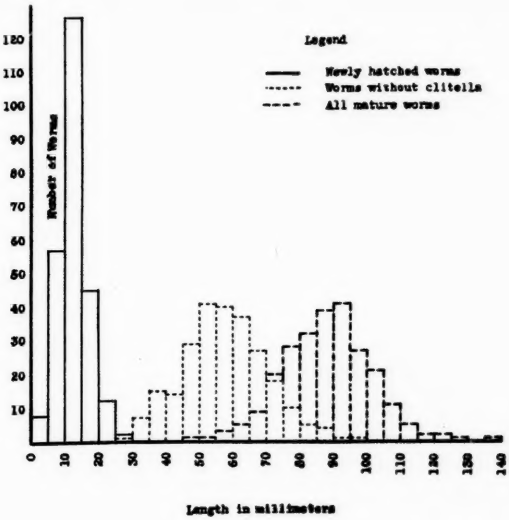
TABLE 7

Lumbricus herculeus Sav.

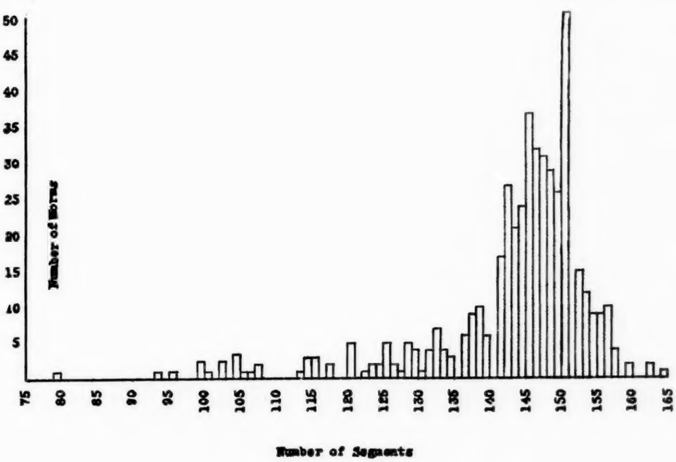
	Range	Mean	S. D.	P. E. of S. D.	P. E. of Mean	S. D./M(100)	Correlation r P. E. of r
Segment length (mm)		1.34					
Worm length (cm)	10–30	19.17	3.077	.067	.094	16.049	
Segment number	79–164	142.71	11.850	.256	.362	8.303	
Segment number in Clitellum	6–8	6.24	0.500	.011	.015	8.019	
Segments anterior to Clitellum	29–32	30.76	0.436	.009	.013	1.416	
Length with segments							+.260 ± .028
Segments in Clit. with anterior seg.							—.629 ± .018
Segments in Clit. with length							+.357 ± .054

Graph 1 presents the frequency distribution for number of segments at all stages of development. Graph 2 shows the frequency distribution for lengths of worms at the various stages of development. Graph 3 is a re-





production of Pearl and Fuller's showing the frequency distribution for number of segments of *Lumbricus herculeus* Sav.



DISCUSSION

In considering the relation of length to number of segments in *Lumbricus herculeus* Sav., Pearl and Fuller point out that the increase in length of an earthworm is due to the operation of two factors: (1) the growth of segments already present, and (2) the addition of new segments. To the predominance of (1) they ascribe the low correlation ($r + .260 \pm .028$) found between length and number of segments. They assume that even after sexual maturity is attained the process of segment formation continues. To this fact they ascribe the skew distribution of the number of segments and conclude that these individuals with lapse of time would add segments at such a rate that they would more nearly approach the mean.

If the data obtained in our research are examined it is found that the correlation between length and number of segments is $+ .43 \pm .0355$ (Table 6) which would seem to indicate a greater degree of relationship between these species characteristics for *Helodrilus foetidus* than is present in *Lumbricus herculeus* Sav. Does this indicate that the number of segments laid down is more important in respect to length of worm in *Helodrilus foetidus* than in the species studied by Pearl and Fuller? Or does it indicate that there is a lower variation in number of segments when maturity is attained? Inspection of Graph 1 shows that in this species we also have a skew distribution. Does this mean that those with fewest segments will add more? By what right may one assume that the skew distribution is accounted for in this fashion, namely, that in some worms the process of segmentation has ceased, that in others it is slow and in others fast? Pearl and Fuller's assumption seems to be that growth (segmentation) tends to change a skew distribution to one more nearly approaching a normal frequency distribution.

A partial answer to these questions is found when one notes the distribution graph for number of segments in

the developmental stage preceding sexual maturity. It can readily be seen that there is no appreciable difference between the range, mean and standard deviation of this selection and those for the mature worms. If one now examines the distribution for the number of segments in newly hatched worms it is apparent that there is still a skew distribution. Comparison with the distribution for mature worms reveals these differences:

(1) The range for the newly hatched worms is much greater—37–136 as compared with 67–124 (Table 1).

(2) There are not only small worms with a lesser number of segments but also ones with a greater number of segments than found in any mature worms.

Considering these facts is it not just as desirable to assume that one may account for the shortening of the range or decrease in variability of number of segments of the mature worms as being due to a process of selection eliminating those individuals deviating from an optimum range, as it is to assume an addition of new segments tending to cut off the lower end of the distribution?

It is shown that there is no significant difference between the means for worms with and without clitella, while one does exist between the newly hatched worms and those without clitella. This does not appear highly significant (7.06, Table 4), because examination of the data reveals that of the small worms 104 individuals exceed the mean of those without clitella, whereas of the latter there are 139 exceeding the mean. Similar comparison between the newly hatched worms and the mature worms reveals that 119 of the former exceed the mean for the latter ,whereas 137 of the mature worms exceed the mean.

The greater dispersion of the newly hatched worms (S. D. 16.95, Table 1) may be accounted for on the basis:

(1) Of natural selection in which both extremes are eliminated;

(2) That some worms normally have a full complement of segments while others normally have relatively

fewer segments upon hatching. This would imply a higher physiological gradient in those having fewer segments so that these individuals gradually overtake the others;

(3) That the artificial environment in a very warm office room with exposure to subdued light and with material different from the normal substances surrounding the cocoons resulted in heightening the activity of developing worms to such an extent that some emerged at an earlier stage of development than would occur under normal conditions. Evidence confirmatory of this is derived from the microscopic examination of the smallest worms. They were: (a) paler in appearance; (b) less active; (c) less clearly segmented near the posterior end, thus increasing chances of error in counting; (d) susceptible to a more rapid disintegration of the cuticle by the formalin preparation.

(4) That the selection involved too small a number of individuals.

The weight of evidence seems to show that selection and not continued segmentation is the cause of lesser dispersion among mature worms than among newly hatched worms.

The means for the body lengths are significantly different (Table 1: Newly hatched worms, 12.32 mm; worms without clitella, 57.64 mm; mature worms, 88.06 mm). An inspection of Graph 2 reveals a much different situation than that found in the one portraying number of segments. The modes are not the same and the overlapping is not so great. Lack of overlapping between distributions for newly hatched worms and for worms without clitella is partially accounted for on the basis of practical difficulties in the collection of the latter. In comparing the means of the lengths for worms without and with clitella it is to be noted that a 34.5 per cent. increase in length has taken place without a significant change in number of segments.

The coefficients of variation in Table 1 show a steady decrease as the worms grow in length, seeming to indicate more rapid metabolic rates in some individuals than in others, thus leading to a closer approximation to type even though the actual range in body length increases with attainment of maturity. These coefficients of variation for lengths contrast with those for number of segments in that the latter show no steady decrease in size, only the first and second stages presenting an appreciable difference. In this case the range in segment number decreases with growth of the worms, the lower part of the distribution being affected most.

The next matter to be considered is the relation existing between the two species characteristics, body length and number of segments. If there were a perfect positive correlation we would of course find that the longer the worm the greater the number of segments. A correlation does obtain amounting to $+.40 \pm .036$ in the mature worms, $+.239 \pm .040$ in those without clitella, and $+.454 \pm .0338$ in the newly hatched worms. The most important factor in the growth of the worm is therefore the growth of the segments rather than the addition of new ones. This is also demonstrated through the 34.5 per cent. increase in the mean for the body length of the mature worms as contrasted with that of those without clitella, with no significant change in the means for the number of segments. It is worthy of note that at an intermediate stage of growth the relationship between these two species characteristics (body length and number of segments) is less than at emergence from the cocoon or at maturity. This would seem to point to a great variability in the rate of growth of the individual segments of the worms.

It should be borne in mind that in all the foregoing discussion the assumption is made that the percentages for the species remain the same throughout the heterogeneous selections. That is, since ten worms out of 250 were *Helodrilus subrubicundus* rather than *Helodrilus*

foetidus it is assumed that a like heterogeneity is found in the other selections. The assumption entails another, namely, that since the worms were collected in the same environment at the same times of the year the times of breeding and growth are similar in both species. It is said that *Helodrilus foetidus* breeds the year round. If, therefore, the other species has a limited breeding season it might well be that there would be no representatives in the non-mature groups, or representation in one but not in the other.

The second objective of the investigation was to secure data upon certain species characteristics of mature members of the species *Helodrilus foetidus* to the end that certain norms be established and the relation between various characteristics be determined. Table 3 presents these values.

It is a matter of interest to observe that there is a much greater correlation between length and number of segments in this species than in *Lumbricus herculeus* Sav. ($+.43 \pm .0355$ in the former and $+.26 \pm .028$ in the latter).

Further comparisons show:

(1) That *H. foetidus* is less than half the length of *L. herculeus* (*H. foetidus* $88.60 \pm .573$ mm; *L. herculeus* $191.71 \pm .94$ mm).

(2) That *H. foetidus* has a smaller number of segments than *L. herculeus* (*H. foetidus* $104.75 \pm .426$; *L. herculeus* $142.715 \pm .362$).

(3) That there is a greater number of segments in the clitellum of *H. foetidus* than in *L. herculeus* (*H. foetidus* $7.4 \pm .028$; *L. herculeus* $6.238 \pm .015$).

(4) That there are fewer segments anterior to the clitellum in *H. foetidus* than in *L. herculeus* (the former $25.629 \pm .029$; the latter $30.762 \pm .013$).

(5) That the correlation, although still negative, between the number of segments in clitellum and number anterior to clitellum is considerably less in *H. foetidus*

than in *L. herculeus* (the former — .40 ± .036; the latter — .629 ± .018).

(6) That increase in length of the worm has no very significant correlation with the number of segments in the clitellum of the species *H. foetidus* (*H. foetidus* + .145 ± .0426; *L. herculeus* + .357 ± .054).

(7) That in *L. herculeus* there is a greater variation with respect to length than with respect to number of segments as compared with *H. foetidus,* the coefficients of variation being: *H. foetidus,* Length 14.8, Segment number 9.3; *L. herculeus,* Length 16.049, Segment number 8.303).

From Table 2 it is apparent that *H. subrubicundus* differs considerably from *H. foetidus* and also from *L. herculeus* in the species characteristics studied. The selection is too small (ten individuals) to warrant much generalization. This species of worm seems to be smaller than *H. foetidus* with respect to body length (average = 75.9 mm). It has, however, a greater number of segments (average = 110.6). It possesses a greater number of segments in the clitellum (average = 8) than either *H. foetidus* or *L. herculeus.* And it has a lesser number of segments anterior to the clitellum than either of the other species.

This research has shown that the only satisfactory way to study changes in growth is to follow a number of worms through from the time of emergence from cocoons until they are mature, noting the changes at various stages of development. Conclusions reached, after a statistical analysis of conditions at any one stage of development, with regard to other stages, are too liable to error on account of omissions of factors which may be highly significant. The technique for measuring a worm without injuring it remains still to be devised, but until one is discovered no positive statement as to the relation of number of segments to growth in body length can be made. All that is possible at the present time is to show in which direction the evidence seems to point. It is the

hope of the writers that in further research they may obtain a definite answer to the problem.

The writers wish to acknowledge their indebtedness to Professor W. M. Barrows for valuable suggestions and to Mr. H. W. Olson for the identification of the species of worms examined in this research.

Conclusions

From the data presented it seems clear that it is not safe to assume that *H. foetidus* continues to add new segments from the time of emergence from cocoons until sexual maturity is reached. Since the data presented by Pearl and Fuller for *L. herculeus* show the same type of skew distribution with respect to segment number in that species as was found in this investigation with *H. foetidus* it seems that such an assumption is not justifiable there also.

It is just as tenable, if not more so, to assume that any difference existing between the means for number of segments of a later stage of development as compared with an earlier is due to selective factors tending to eliminate those individuals which depart from an optimal range. For

(1) There is a greater range in number of segments for newly hatched worms than for mature worms.

(2) This distribution shrinks or is cut off at both ends if that for the mature worms is compared with that for the newly hatched worms.

(3) Although there is a 34.5 per cent. increase in length from the intermediate stage (worms without clitella) to that of the mature worms, there is no significant difference between the means for the number of segments.

(4) Though there is a significant difference between the means for the number of segments in the newly hatched stage as compared with the intermediate selection (Newly hatched, Mean $99.65 \pm .722$, S. D. 16.95;

worms without clitella, Mean 105.5 ± .407, S. D. 9.55; P. E. of difference of means 7.06) this difference is not highly significant when one notes that while there is an increase of 5.85 segments (5.54 per cent. increase) there is an increase in body length of 78.62 per cent.

(5) This slight difference between the means may be accounted for by assuming that: (a) Selective factors have not had time to eliminate many individuals at the lower end of the distribution; (b) the laboratory technique and environment forced some worms to emerge from the cocoons before segment formation was completed.

(6) It seems significant that 104 individuals out of a selection of 250 newly hatched worms exceed the mean for number of segments of the worms without clitella, while 139 of the latter exceed the mean; 119 of the newly hatched worms exceed the mean of the mature worms, with 137 of the latter exceeding the mean.

Apparently the relation between body length and number of segments varies at different stages of development, for the correlation between the two characteristics is + .454 ± .0338 for the newly hatched worms, + .239 ± .04 for those without clitella, and + .40 ± .036 for the mature worms. Obviously the problem can not be definitely settled until young worms with a definite number of segments can be grown to maturity and recounted.

The species *H. foetidus* as compared with *L. herculeus* manifests the following characteristics:

(1) A greater correlation between body length and segment number (+ .43 ± .0355).

(2) A greater number of segments in the clitellum (7.4 ± .028).

(3) A smaller body length (88.60 ± .573 mm).

(4) A smaller number of segments (104.75 ± .426).

(5) A smaller number of segments anterior to the clitellum (25.629 ± .029).

(6) A smaller negative correlation between number of segments in and anterior to the clitellum (— .40 ± .036).

(7) A smaller correlation between increase in body length and segment number in the clitellum ($+.145 \pm .0426$).

(8) A smaller variation in body length in comparison to *L. herculeus* (14.8, coefficient of variation) than in segment number (9.3, coefficient of variation).

Based upon ten individuals the species *H. subrubicundus* is smaller in body length than *H. foetidus* or *L. herculeus* (average = 75.9 mm); it has a larger number of segments in the clitellum (average = 8); a lesser number of segments anterior to the clitellum (average = 21); and it possesses more segments (average = 110.6) than *H. foetidus* but less than *L. herculeus.*

THE TRIPLE-TAIL, *LOBOTES SURINAMENSIS*, ITS NAMES, OCCURRENCE ON OUR COASTS AND ITS NATURAL HISTORY

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THE NAMES OF THE TRIPLE-TAIL

THIS deep, thick, heavily built marine percoid fish is found in all warm seas. Its marked physical make-up is responsible for the interesting names by which it is designated. Of its scientific names, the specific term *surinamensis* was given it by the old German ichthyologist, Bloch, in 1790, because his specimen had come from Surinam in northern South America. The generic term *Lobotes* was assigned by Cuvier in the second edition of his "Règne Animal."[1] It means lobed and refers to the fact that the hinder part of the body is composed of the caudal fin with the soft parts of the dorsal and anal fins projecting backward above and below, all approximately of the same length—giving the body a tri-lobed appearance. This arrangement of the three fins also gives the effect of three tails and hence is responsible also for the best known of the fish's colloquial names—"triple-tail." It is also called "steamboat" by the North Carolina fishermen in allusion to its splashing habits when caught in a seine. A seventeen-pound specimen, which I once caught at Beaufort, convinced me that it was well named. When I attempted to lift it from the seine into the boat, it spined me severely, broke away, and in its flurries to escape deluged all of us who were endeavoring to fish the bunt of the seine. The name "flasher" goes back to Theodore Gill, who in the Smithsonian Report for 1856, p. 260, wrote: "I saw a single specimen [of *Lobotes surinamensis*] in Fulton Market [New York City] last year. . . . The owner called it 'flasher,' but why so named

[1] Paris, 1829, vol. II, p. 177.

I was unable to learn.'' The adult fish is dull colored, even black above, and hence can not flash while dashing through the water as a white or brightly colored fish might. Perhaps Gill misunderstood the fish-dealer, who might have said ''splasher,'' a name which as indicated would fit it admirably.

It is also called ''black perch'' and ''sea perch'' in South Carolina, and ''black fish'' in Louisiana. These names are fairly applicable since it is a marine percoid and generally speaking is black in color as may be seen in Fig. 1—a photograph of a living specimen about two feet long in the New York Aquarium. Off Savannah, Georgia, it is called ''sunfish,'' probably because of its general bodily resemblance to the fresh-water fish of that name and even more because of its habit of lying on one side and basking in the sun as reported by Mr. Padgett and noted later. At Norfolk, Virginia, it is called ''lumpfish'' and ''strawberry bass.'' There seems to be no particular significance in these names and I do not know why they have been applied to our fish.

General Notes on the Occurrence of the Triple-tail on Our South Atlantic Coast

Lobotes is essentially a fish of the warm seas, and as will be shown in this section is found more abundantly as one goes south. North of Chesapeake Bay it is found only as a straggler which has been swept along in the Gulf Stream. It has been taken sparingly as far north as Massachusetts, a fine specimen having been caught off Woods Hole on September 29, 1928, and sent to the Boston Society of Natural History in whose museum it is now on display.

The earliest record for the New York region known to me dates back to Mitchill in 1815, who says that the fish in his day was uncommon in the New York market. The specimen which he described was taken in 1814 in a seine near Powles Hook on the New Jersey shore. It was 13.5 inches long and weighed one pound eleven ounces. At

the present time our fish is taken sparingly in Sandy Hook Bay. Mr. C. M. Breder, Jr., of the New York Aquarium, has listed one or two each year since 1921 as a result of his visits to the pound net fishermen. Probably a few others are taken, but the fish is only a straggler. The captures are practically confined to the warm months of July and August.

Mr. Henry W. Fowler, curator of fishes in the Academy of Natural Sciences of Philadelphia, kindly writes that he knows of but three captures of *Lobotes* on the New Jersey coast: one in 1910, and two in 1913. He has no record of its capture in Delaware Bay or on the Delaware coast.

In the Chesapeake Bay, Hildebrand and Schroeder (1928) say that the triple-tail is not uncommon. Later, when dealing with the fish from the commercial standpoint, they will be quoted in full as to its relative abundance in these waters.

At Charleston, South Carolina, Holbrook in his "Ichthyology of South Carolina"[2] says that the fish is found from June to September, but he gives no intimation as to its relative abundance. Farther south one would expect to find it more abundantly, and Goode (1903) relates that at Jacksonville, Florida, he examined a number of specimens. However, in four seasons' work at Tortugas, Florida, I never saw or heard of the fish. Furthermore, Professor W. H. Longley, whose acquaintance with Tortugas fishes is most extensive, writes that he has in fifteen seasons there seen but three or four specimens, and they not adults.

Later observations at points on the South Atlantic Coast, both north and south of Charleston, give fuller data as to places and relative numbers in which the triple-tail may be found. These data will now be set out.

My personal acquaintance with this fish goes back to the years 1902–11, when as investigator on fishes at the laboratory of the U. S. Bureau of Fisheries at Beaufort,

[2] 1855, p. 169.

North Carolina, I headed the laboratory seining crew or visited the haul-seine fishermen up Newport River almost daily during each summer. During this time I made records of the capture of a number of adult specimens. Since 1911, other adult specimens have been taken, chiefly in the pound net. Still others have been received from fishermen, and more interesting still a number of young fish have been captured. Altogether about twenty-three specimens have been listed in the laboratory records. For some of these, few data are at hand; for others the data are very complete.[3] For ten of these fish we have definite records showing that they run from 18 to 28.3 inches in total length, and for six there are records of weights varying from eleven to eighteen pounds. In addition there are in the collections there three small specimens ranging from 15 to 90 mm in length. The data for these fish will, so far as possible, be tabulated later and then discussed.

From Beaufort southward the triple-tail is found in considerable abundance, and will now be discussed as a game fish. Another section will deal with it as a commercial fish on the South Atlantic Coast.

THE TRIPLE-TAIL AS A GAME FISH ON OUR SOUTH ATLANTIC COAST

My experience of occasional captures of single specimens of *Lobotes* at Beaufort had led me to believe that this fish was not only rare, but also more or less solitary in its habits. This conclusion was confirmed by the statements of other writers—the records everywhere saying "uncommon," "few specimens ever taken," etc. Furthermore, there was, so far as I then know, no record that this fish had ever been taken on the hook. In short, no one knew where the fish was to be found. Hence I was greatly surprised to learn from Dr. Howard A. Kelly, of Baltimore, that Dr. Charles L. Summers, of that city, who

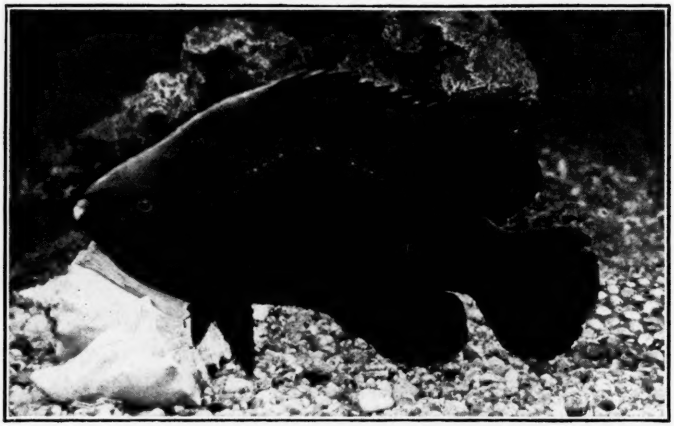
[3] For these and other valuable unpublished data in the Beaufort records, I am under very great obligations to Dr. S. F. Hildebrand, director of the Beaufort Station.

for many years had been fishing at and near Beaufort, had found a locality where triple-tails were taken in numbers on the hook. I urged Dr. Kelly to put this interesting matter on record and this he did in 1923. From his article I excerpt the following facts.

In September, 1923, Dr. Summers found these fish abundant around the hull of an old iron sidewheel steamer sunk during the Civil War about three hundred feet off the shore at a point about eight miles southwest of Beaufort Inlet. Anchoring off this wreck, Dr. Summers (who was seeking "drum," *Sciaenops ocellatus*) could see the triple-tails "disporting vigorously in the surf washing over the deck in about four feet of water at high tide." Going after them with rod and reel, in four days' fishing, nine, fifteen, eight and five (total thirty-seven) fishes, respectively, were taken, weighing from fourteen to twenty-one pounds each after they had been brought ashore more or less dried out. Much tackle was lost both because on being hooked they made for the wreck and fouled the lines and because in the open these powerful fish offered great resistance, leaping from the water like bass, never sulking but fighting from the time they were hooked until landed in the boat. This behavior is at complete variance with the statement of Jordan and Evermann (1896) that these fish are "of rather sluggish habits," as one might readily judge from their short, heavy bodies as seen in Fig. 1.

With the publication of Dr. Kelly's article, the triple-tail entered the lists of game fishes, worthy of the attention of sea anglers. However, fishing at this wreck in later years, only two or three would be taken at a time until on one trip thirteen were caught. Dr. Summers never found them other than at this particular wreck though he fished the coast thoroughly for miles above and below this spot.

So my knowledge of this fish stood until in October, 1927, when Mr. E. A. Simons, of the Charleston (South Carolina) Museum, visited the American Museum and



—*Photograph by E. R. Sanborn*

FIG. 1. A triple-tail in the New York Aquarium (length about two feet). Note the black color characteristic of adults.

asked me what was known about the triple-tail. In return for the little information I could give, he told me that Mr. Harry J. Padgett, master of the U. S. Engineers *S. S. Isondega,* with headquarters at Savannah, Georgia, had had extraordinary success in capturing this fish near the mouth of the Savannah River. Through Mr. Padgett's kindness I am able to set forth the following interesting data.

He catches triple-tails in considerable numbers near Savannah from June till about October 15 (the coming of cold weather), but they are most numerous in August. They are generally found about the piling of beacons or of jetties at the mouths of the rivers or smaller fresh-water streams, usually where there are eddies in which they like to stay. Ordinarily, Mr. Padgett fishes for them about four feet below the surface with a wire snell about three feet long above the hook. "The wire snell is to keep the sunfish [the name common at Savannah] from cutting the line against the barnacles on the beacon piling, since he goes for the beacon when hooked." Mr. Padgett fishes for *Lobotes* not merely for the excellent sport, but he

writes that it is delicious sea-food either baked, fried or in steaks. And Mr. Percy Viosca, Jr., director of fisheries, Louisiana Department of Conservation, informs me that in that state it "is considered one of the most excellent of food fishes and is in much demand when it can be secured."

On August 12, 1926, Mr. Padgett, while off the mouth of the Savannah River, in two hours' fishing, caught eleven triple-tails, three of which weighed 18.5 pounds each, and the whole aggregating 125 pounds. Again on August 24, 1928, Mr. Padgett and his son took a fishing party out in Calibogue Sound near Bloody Point Ranges, off the mouth of the Savannah River. In about an hour and a half the party, despite much lost tackle, caught nineteen triple-tails ranging from 5.5 to 15 pounds in weight, the whole totaling 138 pounds. These fish (and their captors) are shown in Fig. 2. For this photograph and the above valuable data, I am indebted to the interest and the courtesy of Mr. Padgett.

In answer to a recent request for further data as to the occurrence of *Lobotes* in waters under consideration, Mr.

—Photograph by courtesy of Mr. H. J. Padgett

FIG. 2. A catch of nineteen triple-tails, aggregating 138 pounds in weight, taken in Calibogue Sound, off Bloody Point Ranges, near the mouth of the Savannah River, Georgia, August 24, 1928.

Padgett and Mr. Ralph F. Rhodes, of the U. S. Engineer Office in Savannah, have kindly sent me maps of the coastal waters from the harbor of Charleston, South Carolina, to that of Fernandina, Florida. On these they have marked the localities at which they have caught triple-tails or from which they have been reliably reported. Here follow these data.

Triple-tails are found in considerable numbers around the central beacon in St. Helena Sound, formed of the confluent mouths of the Coosa and Morgan Rivers, but are not thought to go much farther in. On the other hand, but few are known to have been taken around the beacons in the mouth of Port Royal Sound. Calibogue Sound at the mouth of the Savannah River has already been referred to, but the two beacons known as Bloody Point Ranges are marked as the best fishing ground along the southeast Atlantic Coast. The fish have even been found as far up the Savannah River as the dock at Quarantine about 4.5 nautical miles inside the beacons named. It is noted that farther south the fish have been seen about the beacons in the mouth of the Altamaha River, and that they are fairly abundant around certain beacons in the center of St. Andrew Sound (the conjoined mouths of Saltilla and Cumberland Rivers). And, last of all, the beacon inside the harbor of Fernandina is marked with the statement: "It is reported that many triple-tails have been taken here."

Consideration of the facts set out above shows that the fish is fairly wide-spread from Chesapeake Bay (where it is taken in pound nets) to Fernandina, Florida. Its occurrence in considerable numbers around beacons and jetties in the sounds and mouths of rivers on our southeast coast ought to attract the attention of sea anglers, since it is evidently a fine sporting fish.

THE TRIPLE-TAIL AS A COMMERCIAL FOOD FISH ON OUR SOUTH ATLANTIC AND GULF COASTS

I have never eaten the triple-tail, and so far as I know, it was never served in "the mess" at the Beaufort Lab-

oratory, where it was my habit personally to partake of various rare fishes in order to get some idea of their palatability. However, Mr. Padgett writes that it is delicious either baked, fried or in steaks.

Its palatability and the fact that it is now known where anglers may find it in considerable numbers would lead to the conclusion that it should become of some commercial value. Hence one who has crossed the lower Chesapeake Bay (say from Cape Charles to Old Point Comfort) and has seen the large number of pound nets operated therein will not be surprised to find that our fish is caught there in sufficient numbers to make it of some commercial importance. Thus Hildebrand and Schroeder (1928) say that while not uncommon:

> The lumpfish is taken in very limited numbers in the lower parts of the bay—that is, at Cape Charles and Lynnhaven Roads to Buckroe Beach, Virginia. No records of the yearly catch of this species are available. It is estimated, however, that the catch for 1922 did not exceed 1,000 pounds, all taken in pound nets [of which the lower bay is full]. A few fish are caught throughout the summer and fall, September and October yielding the largest number. . . . The size of the fish observed in the market ranged from five to twenty-five pounds.

Further information as to the commercial catch of triple-tails on our coast is contained in a letter from Mr. Lewis Radcliffe, deputy commissioner of fisheries. He writes that in Virginia in 1925 the catch was only twenty-five pounds, valued at $4.00; in North Carolina for 1927, 1,013 pounds valued at $27. As has been shown above, the fish is found in considerable numbers in coastal waters south of Beaufort, but the Bureau of Fisheries has no reports of commercial catches in these regions. However, Mr. Radcliffe thinks these totals too small, for he adds:

> In view of these records, it is quite probable that the total commercial catch is somewhat larger than the figures give and that no record has been made of the smaller catches by the fishermen as such, the fish being lumped under the heading of "miscellaneous fishes."

For the commercial catch on the Gulf Coast, the Bureau of Fisheries has no data, but Mr. Percy Viosca, Jr.,

writes that in Louisiana the triple-tail occurs out in the gulf, and that it comes in shore only in the passes at the mouth of the Mississippi River, and in the inlets between the outlying coastal islands and around these islands. Here it is occasionally taken in trawls, but mainly in trammel nets or seines. It is not a very abundant fish, the largest lots reaching New Orleans rarely exceeding 1,000 pounds. These catches are made in the summer.

THE NATURAL HISTORY OF THE TRIPLE-TAIL

Of the natural history of *Lobotes surinamensis* little is known, and that little is contained mainly in unpublished records which fortunately have come to my hand. These will be combined with all the published data and set forth herein, in the hope that thereby interest may be aroused and further information about this interesting fish gotten into the literature.

Breeding season.—Of the embryology of the triple-tail we know absolutely nothing, and even the breeding season is in doubt. Our only clue is the condition of the sexual organs. For males there is but one record. Hildebrand notes a male taken in the pound net at Beaufort on August 5, 1914, as having "testes well developed." Of female fish at Beaufort, he sends two records. The first (size and weight not noted) came from the pound net on June 20, 1916, and is recorded as having "ovaries little developed," as might be expected at that time of year. Of a very large female, 695 mm (27.4 inches) long and eighteen pounds in weight, taken in the pound net on August 25, 1914, he writes: "Roe well developed, eggs quite small, of different sizes, and very numerous. The largest are about one millimeter in diameter, and are probably not quite ripe." This is confirmed by Mr. Padgett, who writes that August-caught female fish are generally full of roe. From this it is fairly clear that the breeding season is in August or later, and that it probably takes place when the fish (presumably) go south. The sexual products are probably cast into the water, and the fertilized

eggs carried hither and yon by currents and tides—*i.e.,* they are presumably pelagic.

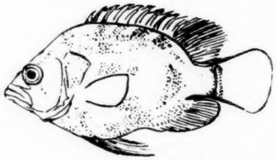
Size.—Of small specimens there is fortunately no dearth, and these are very interesting. For a long time the smallest on record was one of about three inches (76 mm) said to have been collected by Professor S. F. Baird in Tuckahoe River, New Jersey. One slightly larger (3.5 inches), 90 mm, was taken by the writer in Beaufort Harbor in 1902. However, these are by no means the smallest known specimens, and I am fortunate in being able to publish the following table of small sizes ranging from 15 to 161 mm (0.57 to 6.3 inches).

A Graded Series of Young Triple-Tails

No.	1	2	3	4	5	6	7	8	9	10	11	12
Mm	15	18	20	33	48	52	60	76	82	90	118	161
Inches	0.57	0.7	0.75	1.25	1.9	2.06	2.4	3.0	3.25	3.5	4.6	6.3
Locality	Cape Lookout	Port-au-Prince Bay, Haiti	Panama	Port-au-Prince Bay, Haiti	Beaufort	Beaufort	Colon	Beaufort	Beaufort	Beaufort	Port-au-Prince Bay, Haiti	Port-au-Prince Bay, Haiti

No. 1, the smallest known specimen of *Lobotes surinamensis* on record, was taken at the surface of the sea at Cape Lookout, North Carolina, September 13, 1927. It was probably about a year old. Nos. 5, 6, 8, 9 and 10 were taken in Beaufort Harbor and adjacent waters. Some were collected by Dr. S. F. Hildebrand, and all were reported to me through his kindness. Nothing has ever been published on them before. There are two 76-mm fish, one collected at Beaufort in 1902 as noted above. The 60-mm specimen (No. 7) is from the Colon side of Panama. No. 3 (20 mm) is the very closely related (almost indistinguishable) *L. pacificus* from Panama Bay. Both were reported on by Meek and Hildebrand (1925). The little fish from Port-au-Prince Bay, Haiti, were collected

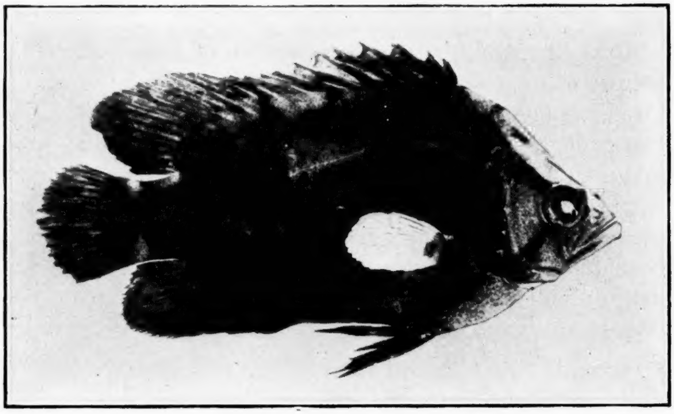
and described by Beebe and Tee Van (1928). Their measurements are "standard," the "over all" measurements (which apply to all the other fish) would slightly increase their lengths. Their 33-mm fish weighed only 1.2 grams. Still smaller is their 18-mm specimen—the smallest *Lobotes surinamensis* of which there is any published record up to now. However, still smaller—and the smallest ever



—Drawing by Mr. W. H. Southwick

FIG. 3. Figure of a 15-mm (0.56-inch) triple-tail taken six miles southwest of Cape Lookout, North Carolina, September 13, 1927.

recorded—is the 15-mm fish from Cape Lookout, North Carolina. It is so small that, in order to have it large enough for any detail to be recognized, it has been drawn 41 mm long and reproduced herein as Fig. 3. While small it is plainly *Lobotes*. Fig. 4 is from a photograph of No. 6, which is 52 mm in total length.

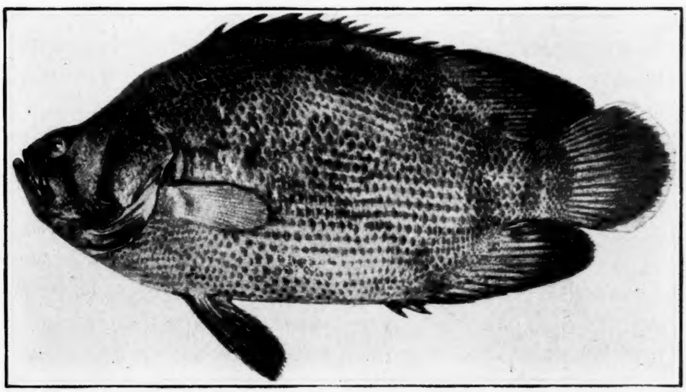


—Photograph by courtesy of Dr. S. F. Hildebrand

FIG. 4. Photograph of 52-mm triple-tail taken at Beaufort, North Carolina, July 27, 1916.

It should be emphasized that save in the matter of coloration (to be studied later) these small fish show no larval structures. All those portrayed herein, and even the little 15-mm fish, are plainly triple-tails—*i.e.*, there is no sign of metamorphosis. On this point I must differ with Jordan and Evermann (1896) who say, "Variable, the young looking quite unlike the adult."

Of forms intermediate between these small, immature fish, and the large adults presently to be referred to, quite a number have been taken but for very few are there any definite data. Hildebrand and Schroeder (1928) record a specimen from the lower Chesapeake measuring 175 mm (6.9 inches). Meek and Hildebrand (1925) figure a retouched photograph, a specimen 185 mm (7.25 inches) long from Colon. This intermediate form is reproduced herein as Fig. 5. It shows the characters of the adult fish



—*After Meek and Hildebrand, 1925*

FIG. 5. A *Lobotes surinamensis*, 185 mm in total length, from Colon, Panama Canal Zone.

very definitely, and is very like the smaller adults taken at Beaufort.

As to maximum size, it is everywhere stated (on the authority of J. E. Tennison Woods) that in the East Indies the triple-tail attains a length of three feet. Certain

data for our southeast Atlantic Coast fish will now be set out in tabulated form since they will give a good idea of the size attained by adult fish. Then record fish (both length and weight) will be noted.

TABLE OF SIZES AND WEIGHTS OF ADULT SOUTH ATLANTIC TRIPLE-TAILS

Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Lengths in mm and inches	457 18	470 18.5	533 21	566 22.3	584 23	635 25	660 26	670 26.4	690 27.2	695 27.4	718 28.3	738 29	900 35.5
Weight in pounds							15.4	12.5		18	16	25	
Locality	Beaufort	Beaufort	Beaufort	Beaufort	New Jersey	Beaufort	Beaufort	Beaufort	Beaufort	Beaufort	Beaufort	Lower Chesapeake	Lower New York Bay

Here we have definite records of thirteen triple-tails ranging from 457 to 900 mm (18 to 35.5 inches) in length, the average being 640 mm (25.2 inches). However, it should be noted that the measurement for No. 13 is "standard"—*i.e.,* to base of the caudal. Had the total or over all length been taken, it would have amounted to about 42 inches (1066 mm). All these fish are from Beaufort (Hildebrand's records and mine) save No. 5 from the New Jersey coast (Fowler), No. 12 from the lower Chesapeake (Hildebrand and Schroeder) and No. 13 from lower New York Bay. I have other incomplete records from Beaufort, but they need not be given since the above are thoroughly representative.

The average weight for the five Beaufort fishes listed in the table is 17.4 pounds. Dr. Summers' fish taken in the open ocean just south of Beaufort Inlet in four days' fishing were thirty-seven, varying in weight from fourteen to twenty-one pounds (lengths not given). Mr. Padgett's first report of fishing near the mouth of the Savannah River gave a total of eleven fish weighing 125 pounds—three of which weighed 18.5 pounds each. His second ac-

count is of the taking of nineteen triple-tails, ranging from 5.5 to 15 pounds and aggregating 138 pounds.

In Louisiana waters, the average weight, according to Mr. Viosca, is between seven and fifteen pounds; "large" specimens, however, weigh between twenty-five and twenty-eight pounds, and the maximum is about thirty pounds in weight and three feet in length.

Record fishes for size and weight, not only for our coast but for any and all waters so far as I know, will now be noted. No. 13 in the above table was obtained in 1925 from a pound net fisherman in lower New York Bay by Mr. C. M. Breder, Jr. This fish was kept for some time (until its death) in the New York Aquarium, where it attracted much attention. Its length (standard) of 35.5 inches is to the base of the caudal, but its total or over all length would have been about 42 inches (1066 mm). It is the largest triple-tail on record, and had its weight been taken this would have been the greatest known. The heaviest fish known to me to have been put on the scales is one of twenty-six pounds (length not recorded) taken by the late Russell J. Coles at Cape Lookout, North Carolina—where the smallest (15 mm) was also caught.

Coloration.—In my early acquaintance with the triple-tail, I found difficulty in reconciling with the descriptions in the books the fact that my 3.5-inch Beaufort specimen had white pectorals and a wide white margin on the caudal. The only explanation I could give was that this was a juvenile coloration. This was correct, as has since been found from a study of the coloration of other young and small specimens. In general it may be said that there is considerable variation in the coloration of juvenile, half-grown and adult fish, and even in fish of the same size.

For juvenile or small fish, the color may in general be described as follows: The body is brownish or greenish—darker at the back and shading off to lighter downward—with yellowish or grayish blotches. The fins are generally of the same color as the body, except the pectorals which are whitish translucent. The caudal has a variable

broad marginal band of the same color as the pectorals, especially marked in the young. The dorsal and anal fins have the general body color but are narrowly margined with lighter. Two narrow streaks on top of head between the eyes extend to nape. One streak runs from posterior margin of eye upward to nape, and another one runs from posterior margin of eye downward to the lower angle of the preopercle. These streaks are not very sharply defined. In very small specimens the brownish pigment tends to become concentrated in the centers of the scales and in certain areas, forming ill-defined cross bars and narrow longitudinal streaks along the rows of scales. There are frequently several dark spots on base of soft dorsal and anal, the latter sometimes distinctly ocellate.

The 15-mm specimen from Cape Lookout, shown in Fig. 3, is so small that only the light and dark areas can be made out. No color notes for it when fresh are on record. It is significant that not only the whole pectoral but also the *whole caudal* are white. It would be interesting to know if other specimens as small as this have white caudals. Beebe and Tee Van note that their 18 and 33 mm fish (standard length) had each a caudal with "very wide translucent terminal band."

Fig. 4 is from a photograph of the 52-mm specimen. Here the coloration is shown very accurately, since the specimen had been in alcohol too short a time (less than a week) for the color to have been materially changed. The pectoral is still transparent save at the base; the caudal, however, lacks the marginal white band. But this little fish is plainly *Lobotes*—it can not be any other fish.

Fig. 5 portrays a considerably older fish, which is beginning to take on the adult coloration. It is copied from Meek and Hildebrand's retouched photograph of a 185-mm (7.25-inch) specimen from Colon, Panama. This fish still retains the terminal white margin of the caudal, while more noticeable than in Fig. 1 of the same plate are the dark spots at the base of the soft dorsal.

That the juvenile colors above described are sometimes carried over into adult life, or may be revived on occasion, is recorded by Hildebrand of an adult male, 690 mm (27.2 inches) in total length. This fish was taken at Beaufort on July 11, 1913, and as this is (presumably) about the beginning of the breeding season for these fish in these waters, it may be conjectured that this was the nuptial coloration. (Unfortunately no examination was made to ascertain the condition of the testes.) Here are Hildebrand's carefully made but hitherto unpublished notes.

General color bronze and green above and on sides, lighter below. Closer inspection shows area around jaws and gills to be yellowish, caudal dark blue-green, dorsal and anal pinkish-green and lighter than caudal. Pectorals yellowish. Pre-dorsal area a marked peacock color. Center of scales on back and sides a deep bronze-green with margin of blue. The bronze and green on back are especially marked, although so dark as to appear almost blackish.

As the fish grows older it becomes darker and rather more uniformly colored. Of the adult fish Jordan and Evermann (1896) say "Blackish above, becoming silvery gray on the sides; often blotched and tinged with yellow." Of Beaufort specimens, Smith (1907) says, "Dull black above, silvery gray on the sides and below; fins dusky; sides and fins sometimes with small yellowish blotches," while Hildebrand and Schroeder (1928) record that "Large fish examined by us in Norfolk fish markets were black everywhere on body and fins."

Such is the color of the fish (*circa* twenty-four inches long) photographed in an exhibition tank in the New York Aquarium as shown in Fig. 1. This fish's body, unfortunately, was not in exact plane with the plate in the camera. Mr. Breder tells me that the triple-tails up to about two feet long caught in Sandy Hook Bay are more or less mottled, above that size they are darker, and that they tend to become black when kept in the aquarium, as was the fish referred to. At this late day I can not clearly recall the color of my Beaufort specimens, but my recol-

lection is that at any rate they approached in color the fish in the aquarium.

From the data given it is plain that there is much difference in the coloration of the fish of any stage taken in different localities, that the juvenile coloration varies greatly from that of the adults and that the colors of the adults tend to become darker (grayish to black) as they grow older. It seems not improbable that Hildebrand's July-caught adult male was taking on the (presumed) nuptial coloration.

Food.—As to its food in nature we know little. One of 27.2 inches dissected at Beaufort contained five small menhaden (*Brevoortia tyrannus*). Beebe and Tee Van (1928) found the vertebra of a fish in their 118-mm (4.75-inch) specimen. Goode (1903) says that it feeds on small fishes and mussels, and that it readily takes the hook when this is baited with clams or with shrimp. Dr. Summers used cut mullet and squid for bait. Mr. Padgett, who has had more experience than any one else, uses shrimp, large prawns or small mullet. Specimens in the New York Aquarium are fed on shrimp, clams or cut fish. They are rather slow to feed, but when once induced to do so, they, generally speaking, eat the food fed to the other fish in the tanks with them.

Habits.—As has been noted above, my scattering catches at Beaufort (six in ten years), despite daily seinings or visits to the haul net fishermen, led me to believe that the fish was solitary. This, however, has been discounted by the experience of Dr. Summers and Mr. Padgett as set out above. It is an undoubted fact that this fish goes in schools under favorable conditions. Dr. Summers could see numbers of triple-tails about the old wreck described above. The details of the numbers taken by Dr. Summers and Mr. Padgett are set out in a previous section. Further, the latter says, "They are often seen in numbers floating at the surface on sunny days, when they are easily caught." He has sometimes seen them in schools of about a hundred in number, and he concludes

by saying that at such times "I have known them to come up close enough to the boat, floating on their sides [whence probably one of their common names, sunfish], so that one could easily strike them with an oar."

In this connection Mr. Breder tells me that their specimens in the aquarium at times seem to avoid "riding on an even keel," that they will lie for hours at an angle of 45° to the perpendicular but without any perceptible fin action. He thinks that this position is voluntarily assumed and that it is possibly done so that the fish may keep an eye on what is going on above the tank. I have elsewhere recorded similar behavior in the gray snapper (*Neomaenis griseus*) at Tortugas, Florida.

It is interesting to note that the triple-tail seems generally to be found about wrecks, the supports of beacons, the piling of jetties, sea buoys, etc. Then one asks, "Why?" Hardly for protection, since in our waters sharks are too scarce and larger teleosts too few to count much as enemies. "For food?" Possibly. Yet on this point little can be said since our knowledge of what its natural and normal food is in the wild state is almost nil. "For shade and shelter?"—probably. This is the explanation that strikes me as most plausible. In this conclusion I am influenced by the behavior of the fish noted above in the wild state and also in captivity. In the tanks of the New York Aquarium, triple-tails have proved to be timid, easily frightened, ordinarily inactive and fond of the shade. In this aquarium, above and behind each exhibition tank is a storage tank which at certain times of day casts a shadow in the rear of the lower tank. Into this shadow the triple-tail invariably retreats. To lure him out into visible range, a piece of heavy board, twenty or thirty inches long, is anchored where wanted, and under this the fish will contentedly take his place in full view of visitors. This I, myself, have seen a number of times.

That this is true of the triple-tail in the open sea is confirmed in a recent communication from Mr. Padgett in which he says, "I have seen numbers of this fish in the

vicinity of floating buoys, and also near the surface of the water in fairly large numbers around other floating objects, such as packing boxes and other débris drifting around in the ocean near the coast. When found under these conditions, the fish are easily frightened and disappear as soon as a boat approaches." Since this section was written, I have received from Mr. Percy Viosca, Jr., the following confirmatory account of the habits of the triple-tail as observed on the Louisiana and Mississippi coasts:

> It is noted for its habit of lying slightly tilted on one side near the surface of the water. It is particularly fond of lurking in the shadows of floating drift, and along the outlying islands numbers of them can sometimes be seen in the shadows of fallen bushes projecting over the water where the depth is from two to three feet.
>
> On the Mississippi coast, off Pascagoula, enterprising fishermen place the tops of pine trees out in the gulf near the islands, and fish around these with dead shrimp about eighteen inches below the surface of the water, using a long cane pole and a float. The pine tops are approached cautiously with a skiff and the bait is cast around the edge of the pine tops by means of the long pole. Sometimes as many as two or three fish are caught around each pine top.

That this habit of hiding is true of young fish as well is confirmed by Beebe and Tee Van, in the paper previously referred to. They say:

> The two smaller fish were taken by scooping up Sargasso weed in a hand net, the fish being found among the fronds. . . . [They] were taken under separate patches of Sargassum, so that there can be no doubt that the young are to be found under these conditions.

While this is just what one would expect of young fish, still it ties up closely with the behavior of the adults both in captivity and in the open sea.

How these differing observations on the habits of the triple-tail are to be reconciled is not apparent. They are facts which must stand while we wait for further and more complete knowledge of the activities of this very interesting fish. It is my hope that the publication of this article may arouse interest and lead to further study of the triple-tail.

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THE INFLUENCE OF ECOLOGICAL FACTORS ON THE SIZE OF POPULATION

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THE problem of ecological distribution of organisms seems to be one of the least investigated problems of quantitative biology. It is a branch of the biology of population (*sensu* Pearl) (Pearl, 1928) and can be divided into two parts: (1) An exact study of the relationship between an abundance of given species (size of population) and ecological factors in natural conditions, and (2) an experimental study of the influence of ecological factors on the population size. We have analyzed the first of these questions in a special paper on the ecological distribution of Orthoptera (Gause, 1930). Our conclusion was that the relationship between an abundance of a species and ecological factors follows the law of Gauss. This enabled us to find the mean value for each species, which characterizes the average ecological conditions, and standard deviation, as an indicator of the ecological plasticity of the species. It was found that the ecological plasticity of a species is closely connected with the place occupied by its average conditions on the ecological scale. The more the average conditions of this or that species deviate from the middle of the scale, the less is their ecological plasticity. The object of this paper is to investigate the second group of questions, that is, the influence of ecological factors on the size of population, and to generalize the facts and data obtained in the course of the field as well as of the experimental study of the ecological distribution.

We will now examine the influence of two ecological factors, the quantity of food and the temperature, on the size of population. In both cases we are trying to express in a mathematical form the experimental data published by Chapman (1928) and Terao (1928). The first

of these authors deals with the population growth of the flour beetle (*Tribolium confusum*) in different quantities of food. He has published considerable experimental material without any attempts to analyze it mathematically. Terao studied the influence of temperature on the growth of population of the water-flea (*Moina macrocopa*). He fitted the logistic curves, representing the population growth in different conditions. The relation between the size of population and the ecological factor, however, did not interest the author.

An experiment on the influence of the quantity of food on the size of population will be the first thing we examine. We have taken the data on the population growth of *Tribolium confusum* in 16, 32, 64, and 128 grams of food (Table IV, p. 118 of Chapman's paper). These data based on considerable material are most convenient for mathematical calculations. In every case the experiment has been made on the following lines: A pair of newly emerged beetles has been placed on the food, wheat flour, which has been placed in quantities of different weight, but always in a layer two centimeters deep. The experiment has been made at a constant temperature of 27° C. and with a uniform moisture. On an average the observations have taken place every seventeenth day, when the food has been renewed and the numbers of eggs, larvae, pupae and adult beetles have been counted. This gives the size of population at different moments of its development.

The biology of the population growth of organisms has been recently investigated from different points of view by R. Pearl (1925), who succeeded in proving that the mathematical expression of the population growth is the logistic function. Recently the logistic function has been successfully applied to a whole range of biological problems (Alpatov, 1929). We fitted the curves of the growth of population in different quantities of food by using the logistic function, and obtained the data given in Table I. This table shows that the duration of the population

TABLE I

GROWTH OF POPULATION OF THE FLOUR BEETLE (*Tribolium confusum*) IN DIFFERENT QUANTITIES OF FOOD

Quantity of food in gr.	Upper asymptote	Equation of the logistic curve	The point of inflection of the curve in days
16	650	$y = \dfrac{650}{1 + e^{4.26903 - 0.09057x}}$	47.135
32	1025	$y = \dfrac{1025}{1 + e^{4.31967 - 0.08289x}}$	52.113
64	1750	$y = \dfrac{1750}{1 + e^{4.94020 - 0.08217x}}$	60.121
128	5000	$y = \dfrac{5000}{1 + e^{5.19022 - 0.05415x}}$	95.849

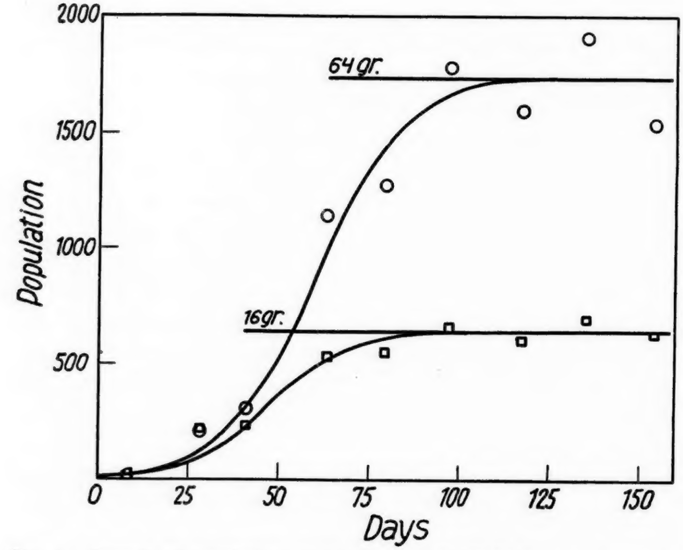

FIG. 1. Growth of population of the flour beetle *Tribolium confusum* in 16 and 64 grams of flour.

growth increases together with the increase of the quantity of food. As to the other characteristics of the curve, the upper asymptote is of especial interest for us. It shows us the asymptotic population under different conditions (Fig. 1). Evidently the asymptote is a good characteristic of the size of population in equilibrium, which corresponds to an abundance of species in nature. In field work, however, we can evaluate the abundance with but little accuracy, and study its relation not with separate ecological factors, but only with the microclimatical complexes, whereas in the simplified conditions of an experiment we are able to study with great accuracy the relationship between the size of population and the ecological factor.

The correlation between the asymptotic population and the quantity of food is represented in Fig. 2. An absence of optimal conditions and the decrease of the size of population if the conditions deviate from the optimum constitute the peculiarities of this factor. We have here the simple exponential relation between the variables, which

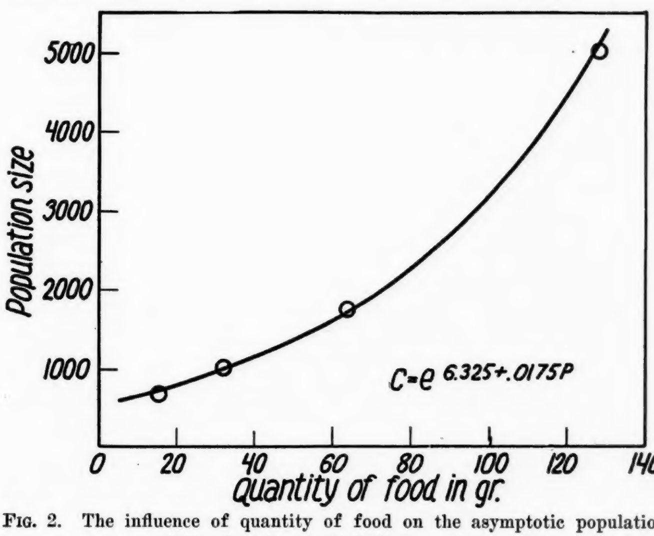

FIG. 2. The influence of quantity of food on the asymptotic population of the flour beetle *Tribolium confusum*.

can be considered as a special case of Pearson's generalized curve of probability.

As to the influence of temperature on the size of population, the experiment has been made as follows. Parthenogenetic females of the water-flea have been taken from nature and placed in vials with water containing unicellular organisms, which served as food for the water-fleas. Two cultures have been placed in the thermostat, in an average temperature of 19.8° C., two in one of 24.8° and four in one of 33.6°. Every day the water-fleas have been counted and fresh food-water has been given. Fig. 3 represents the correlation between the asymptotic pop-

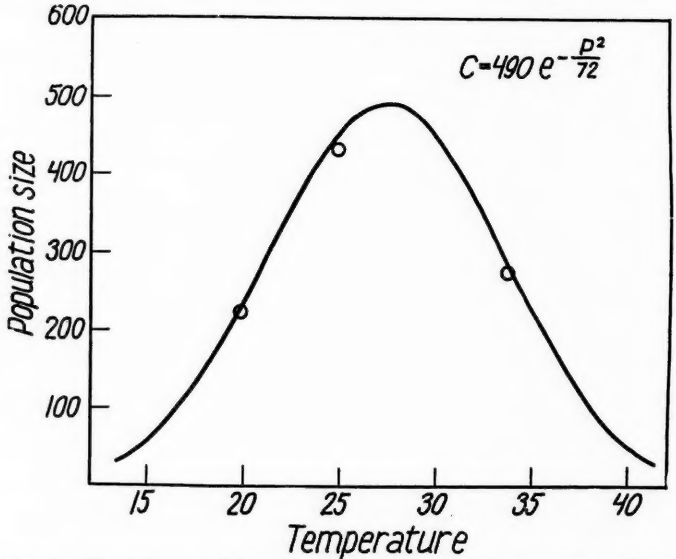

FIG. 3. The influence of temperature on the asymptotic population of the water-flea *Moina macrocopa*.

ulation and temperature. We have here the maximum population size in optimal conditions (which in this case coincide with the average ones), the size of population decreasing regularly when the conditions deviate from the optimum. The normal curve in general represents a good approximation to empirical data.

On the basis of the analyzed material we can endeavor to generalize the relationship between the size of population and ecological factors. Following Lotka (1925) we can write an equation of the kinetics of evolution, which represents the changes in time of one of the components of the system:

$$\frac{dX_1}{dt} = F_1 \ (X_1, X_2, \ldots X_n; P_1, P_2, \ldots P_j; Q_1, Q_2, \ldots Q_k)$$

where $X_1, X_2, \ldots X_n$ represent the masses of the given component and of others, $P_1, P_2, \ldots P_j$ are parameters defining the conditions in which the growth of the component takes place and $Q_1, Q_2, \ldots Q_k$ are parameters defining the character of several components. Thus the rate of growth of one of the components of the system is a function of a whole series of parameters. If we apply this equation to our example we obtain:

$$\frac{dX}{dt} = F(X, P).$$

The rate of the population growth is a function of the size of population and of the ecological factor. The simplification of the conditions in the experiment enables us to neglect other parameters.

In a case of equilibrium $F(X, P) = O$ and $X = C$, that is, population reaches an asymptotic value. The asymptotic population depending from the ecological factor we can express in the following form:

$$C = F_1 \ (P).$$

The basis of the relation between the size of population and the ecological factor being the law of Gauss (generalized by Pearson), we can write for a case of normal distribution:

$$C = \frac{N}{\sqrt{2 \pi} \sigma} e^{-\frac{P^2}{2\sigma^2}}$$

where σ is the standard deviation of the variation series constructed with asymptotic populations, which they reach in these or those conditions.

Taking the generalized equation of the curve of distribution we obtain the following expression for the connection between the size of population and the ecological factor:

$$\frac{1}{C}\frac{dC}{dP} = \frac{P - a}{b_0 + b_1 P + b_2 P^2}$$

where the denominator on the right side is F_1 (P) fractioned according to Maclaurin's series, and the constant *a* shows that the beginning of coordinates can be chosen at will. By using these equations we can determine the size of population with any given value of ecological factor.

The rules here established might be of great use in applied biology. The possibility of predicting *en masse* appearance of injurious animals under certain ecological conditions can facilitate the control work of pests of agriculture. The same holds true concerning wild animals useful to man.

The author is deeply indebted to Dr. W. W. Alpatov for critical suggestions and interest in the present investigation.

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ABNORMAL HERPETOLOGICAL SPECIMENS FROM SYRACUSE, NEW YORK

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In the course of recent field work three unusual and interesting animals have been taken, the first being absolutely unique as far as can be determined from published records consulted.

I. A SPOTLESS SPOTTED SALAMANDER, AMBYSTOMA MACULATUM (SHAW)

Near Fayetteville, New York, a suburban village eight miles east from the center of Syracuse, lies a valley whose northern boundary is a steep hillside, stone-littered and heavily wooded with a variety of deciduous trees. An abundance of flat rock and fallen timber together with a rich humus and moist soil make this slope an excellent collecting ground for salamanders and snakes. Below, there is a level meadow with a series of cattail marshes at its upper or eastern end, and from these rises a small stream, in the course of which a good-sized, elongate pool occurs, averaging three feet in depth and ten in width. Throughout the brief extent of the brook, but especially concentrated in the pool, there is a wealth of life, among which water-snakes, green frogs, newts, spotted salamander larvae, caddis-fly larvae, water striders and pond snails stand out conspicuously. On the hillside the forms most frequently collected are milk, DeKay's and garter snakes, newts in the land stage, spotted, slimy, red-backed and dusky salamanders, and a host of worms, sow-bugs, myriapods, and cryptozoic insects, especially ground-beetles and ants.

The specimen of spotted salamander in question was discovered on May 14, 1928, beneath a flat rock about half way up the slope opposite the pool, while another and entirely ordinary individual of the same species was

found when a stone just alongside was turned. Both examples were kept alive for some time in a laboratory terrarium, but were later preserved and deposited in the U. S. National Museum, where the normal *Ambystoma* received number 76136 and the abnormal one number 76137. The typical animal was kept with the other both in the living and preserved state, because taken at the same time and place and for purposes of contrast, since it was in every way characteristic of the species.

The two captives were as alike as two peas except in the matter of coloration. They both agreed in all the well-known external structural features of *Ambystoma maculatum,* having the correct symmetry and proportions of the body and of its parts; number, length and structure of the digits; distribution of glandular pores; the skin grooves in relation with the jaws, orbit, parotid region and gular fold; a strong mid-dorsal crease, and eleven costal grooves. The detailed description of Cope (1899, pp. 56–59) applies equally well to both save for the coloration of the unique specimen. The length of the normal example was 156 mm, tail 68 mm; length of the abnormal one 162 mm, tail 80 mm. Both were adult males.

In the typical individual there were the regulation large, bright yellow spots scattered over head, body, limbs and tail, on a jet-black ground. In the other animal, the color above was everywhere a deep uniform black, lustrous when wet, and imparting a very handsome appearance. Below, the skin was somewhat lighter, being dark plumbeous gray. Along the dorsolateral margins of the tail were tiny whitish dots surrounding the pores of mucous glands, and in close view these stood out more conspicuously than in the normal form, owing to the absence of other coloration.

And yet this melano was not altogether spotless. When minutely examined, three of the customary blotches could be made out, all located at places where large, bright spots appear on the average salamander. One of these could be noted in the middle of the left shank, on the

dorsocaudal surface, and was of fairly good size, though smaller than is usual and of a faint yellow tint. The others were very small and faint indeed, requiring careful scrutiny to determine; one was situated just below the knee on the dorsal exposure of the right shank; the other appeared on the dorsolateral surface of the left parotid region. The arms, body and tail were immaculate.

When the animal was preserved in formalin these three spots became somewhat more prominent than in life, even though their color was slightly bleached, as are the large spots of a normal *A. maculatum* when thus treated. The skin took on a leaden gray hue and through it could now be made out a very fine stippling of white, giving a pepper and salt pattern, and due to the action of the preservative on the mucous secretion contained within the dermal glands.

A year of desultory correspondence and literary search has failed to bring to light any records of complete melanism in this species, and in fact has shown that abnormalities in coloration among amphibians are rare and little understood. Britcher (1899) describes the finding of a batch of about one hundred albino eggs of the spotted salamander, but these developed the typical pigmentation as they matured. Dr. Frank N. Blanchard, to whom I am indebted for several references, sent me a partially melanistic preserved example of this species, collected in White's Woods, Ann Arbor, Michigan, March 26, 1927, which I have since deposited in the U. S. National Museum, where it was given number 80337. It has somewhat less than the usual number of spots, all of which are smaller than customary, imparting a much blacker appearance than that of normal specimens, but nevertheless is spotted all over and is by no means as striking in respect to color abnormality as is the Syracuse salamander. Other references encountered had to do with experimental work in the modification of pigmentation, none of which seem to the writer to have any particular bearing on this case.

Considering that such a melanistic state in *Ambystoma maculatum* has not hitherto been reported, in spite of wide use of the species in biological investigations, the condition must be of very rare occurrence. It thus obviously can not be genetically interpreted as due to chance combination of multiple color determiners, and is too unique to permit of explanation by the smallest known percentage of crossover. Reversion seems unlikely, as jet black is not at all common among the caudata, especially the more primitive types. A new mutation, of unknown cause and behavior, appears to the writer as the most likely speculation; we know very little indeed about either melanism or color factors in salamanders.

II. A White-Bellied Red-Bellied Snake, Storeria occipito-maculata (Storer)

On July 10, 1928, the weather being clear and hot, this reptile was found beneath a piece of composition roofing which had been left lying in some deep grass along the eastern edge of the Syracuse University campus, at the foot of a hill famous locally as Mount Olympus. Numerous ground beetles, crickets, myriapods and sowbugs were noted under this and other pieces of débris, rocks and small logs in the immediate vicinity. When viewed from above during the capture, this snake appeared as a regulation individual of its species, but upon picking it up and turning it over to exhibit the red underparts to a group of students, the writer was astonished to find the belly an immaculate white. Laboratory examination showed further irregularities present in the head shields. Systematic description of the snake follows.

Body: head distinct from neck, and body quite stout; the specimen being a female distended with embryos: dorsal body scutes strongly keeled and in 15 rows: ventrals 127, the last being incomplete on the right side so that the last two resemble a divided plate: anal divided: subcaudals 42, in two rows: tail short but tapering uniformly to a fine point: L. 279 mm, T. 53 mm.

Head, normal shields: rostral and frontal single, internasals, prefrontals and supraoculars paired: nasal divided, the naris being located almost wholly in the prenasal, which is smaller than the postnasal: loreal absent: preoculars two, subequal: supralabials six, third and fourth entering eye, which is large; fifth supralabial very long and sixth next longest: infralabials seven, fifth the largest: pregenials longer and larger than postgenials, behind which comes the undivided first ventral.

Abnormal conditions were presented by the postoculars, temporals and parietals. The postoculars numbered three, subequal, on each side, instead of the regulation two; those of the left side were mutilated by some accident antedating capture. The temporals of the right side were normal, with the formula 1–2, the first being very large and long, very much pointed posteriorly and extending over nearly all of the sixth supralabial. On the left side there was a small accessory shield placed between a parietal which was hollowed-out along the middle of its lateral border, and the upper temporals. The anterior half of the lateral margin of this extra plate, directed caudoventrad, was in contact with the first temporal over the third-fourth of its extent; the posterior half, directed caudodorsad, abutted upon the upper temporal of the second row.

Coloration, living: dark pearl gray above, with a lighter vertebral band on the three central rows of scutes, bordered on each side by a darker brown stripe one scute wide. The lateral thirds of the gastrosteges, visible from above, darker gray, giving the appearance of a dark stripe along the flanks. Abdomen milky white. The three yellowish-brown nuchal spots diagnostic of this species were present and fairly well pronounced.

When preserved in formalin the upperparts took on a general tone of chestnut brown rather than gray. Examined attentively, and especially when observed through a binocular microscope, the detailed pattern was as follows: the dorsal pigment was yellowish-brown, minutely

and irregularly flecked on each scute with dark gray; about one half of the exposed surface of each scute was bordered around its periphery by a wide band of the gray color; the bulk of the fifth supralabials as well as the three nuchal blotches were yellowish-brown, on account of the suppression of the gray in these places. The flanks were so heavily speckled with the gray as to give the appearance of a nearly solid gray band, but this stippling thins out ventrally so that it disappears or is very sporadic and rare on the middle third of the gastrosteges. A narrow gray band, however, continues across most of the ventral plates in their posterior halves. Both series of labials, chin and throat speckled with the gray, more heavily forward, the chin being more gray than white, but fading out posteriorly. Head shields above chestnut brown, irregularly blotched with fuscous.

Not long after its capture this specimen shed the skin and appeared a rich and lustrous gray above, while on the abdomen the faintest tinge of opalescent rosy pink was evident. The color below would still be described as white, but it was interesting to observe this transient evidence of the normal coloration. Although in excellent condition when captured and appearing to thrive well in the terrarium all summer, six imperfectly formed young were aborted on August 6.

This species is subject to considerable variation in details of the dorsal aspect as well as in the actual color itself of the belly. Extracts from the descriptions of various observers give the dorsal ground color as olivaceous, olivaceous-gray, gray, dark gray, grayish-brown, chestnut-gray, chestnut-brown, brown, almost black. A lighter vertebral stripe is usually but not always present. This is in most cases bordered on either side by a row of fine dots that are dark brown, black, or even sometimes white, and which occasionally run together into a longitudinal stripe; but some snakes are uniform brown above with no vertebral or lateral stripes or dots. The flanks seem always to have a fairly wide band of bluish- or slate-

gray. Abdomen salmon, salmon-pink, brilliant red, bright red, bright brick red, crimson, rich crimson, vermilion. These phrases present both a general scheme of color and pattern, as well as the commoner range of variation.

Cope (1900, pp. 1004–5) lists markedly divergent individuals from Charleston and Anderson, South Carolina, in which the body is dark slate-blue except the middle third of the abdomen, which is yellowish-white. One of his specimens from Pittsburgh, Pennsylvania, is similar but with a light chestnut vertebral stripe, contrasting strongly with the ground color. Another, from Johnsonburg, Elk County, Pennsylvania, is very dark brown with the dorsal band obscure and the undersurfaces black; the throat and chin are whitish and the black is darkest in a row of spots on each side of a dozen anterior gastrosteges. Pickens (1927, p. 112) mentions "a slate-blue, yellow-bellied example from Miss Charlotte Paine, Anderson (S. C.) U. S. N. M.," presumably the same snake listed by Cope. These are the only references to a white abdomen known to the writer, nor does the literature on this snake mention such aberrant head plates as are shown by the present specimen. The case is not as unique as that of the black *Ambystoma,* but nevertheless seems worthy of recording. The value of such an individual as an exhibit is largely vitiated by the fact that the normal red color of the abdomen is bleached by preservative to a soiled grayish- or yellowish-white, hence the white-bellied example presents no unusual coloration after being a short while in formalin. This specimen was sent to the U. S. National Museum and has received number 76135.

III. An Albino Mudpuppy, Necturus maculosus (Rafinesque)

During the first week of March, 1929, Mr. Fred R. Main, of Syracuse, was fishing through the ice of Oneida Lake near Shackelton's Point in about fifteen feet of water, and caught a perfect albino example of this salamander.

Though a large hook was embedded firmly in its throat, the animal lived for some time and created quite a bit of interest locally, being exhibited in the pharmacy window of Mr. E. H. Newell, who kindly presented the specimen to the writer. It has since been deposited in the U. S. National Museum, and numbered 80336. It may be noted as a rather amusing coincidence that all three of the abnormal animals which form the subject of this paper have specific names based on the Latin *maculatus*, spotted, and that the two amphibians fail to live up to this appellation.

The *Necturus* was an adult female measuring as follows: head, ventral aspect from lip to gular fold, 30 mm; trunk, 145 mm; tail, 65 mm; total length, 240 mm. The living creature was a very beautiful object indeed, being entirely rosy white, with bright red eyes and external gills. This rosy hue was due to capillary dermal circulation, and the only pigment appearing at any place occurred on the ends of the digits, which were tipped with chocolate brown. Not even a trace of spotting could be detected. Preserved in formalin, the animal became of the intense whiteness of the lily, including the eyes and gills.

Several zoologists and sportsmen who were consulted in person or by correspondence had vague recollections of reading or hearing about such albinos, and it seemed to the writer that he had run across an allusion to the condition here and there in print, but upon trying to trace these ideas down to definite citations, little success accrued. Apparently albinism in this species is not unknown, but only one published record came to light: Cahn and Shumway (1926, p. 107) collected two white *Necturus* larvae in Waukesha County, Wisconsin. These were preserved at the 18-mm stage, and were immaculate white on a pale yellow yolk sac. In view of the fact that the albino eggs of *Ambystoma* mentioned above gradually darkened with age, it can not be positively asserted that these white waterdog larvae would have remained colorless through-

out life, though it is very probable they would have done so. The present example is the only recorded adult known to the writer. The same authors also mention finding a number of *Necturus* larvae that were so very dark brown as easily to pass for black, and Cope (1899, p. 26) describes an entirely black individual from Ontario, Canada. The mudpuppy thus appears to be subject to more striking color variations than is usual in the caudata, but such extreme conditions in any member of the group are evidently very rare.

Addenda

While the foregoing article was in press another paper on albino salamander eggs has appeared (Cahn, A. R., 1930). Six clusters of these eggs were found, totaling 71, and yielding white larvae. Observations are at present continuing on the amount and rate of pigmentation developing as these larvae mature, some being raised in normal illumination and others in total darkness.

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SHORTER ARTICLES AND DISCUSSION

A MUTABLE MINIATURE GENE IN *DROSOPHILA MELANOGASTER*

DURING the past three years I have several times found in X-rayed material flies that showed characters of the so-called eversporting type. The most common of those met with is the one that gives rise to mottled-eyed flies. Some of these have been developed into stocks and given to Mr. H. R. Bolen, who is making a special study of their genetics. Most, if not all of the mottled-eyed forms are the result of chromosome abnormalities, chiefly translocations. I have recently found a case of a mutable miniature gene that merits recording. The origin and the history to date of this case are as follows:

The original fly arose in an experiment in which virgin females were X-rayed and then crossed to yellow white lozenge miniature males. The females used belonged to a "duplication" stock, in which one X-chromosome carries the recessive factors yellow and scute, and the other X-chromosome has, in addition to yellow and scute, a small piece of the left-hand end of another X-chromosome attached to its extreme right end. This fragment carries the normal allelomorphs for yellow and scute. The sons of such females will be of two classes, namely, yellow scute and wild-type (duplication type). Mass cultures were made with the treated females, mated as stated above. These cultures yielded a total of 3,678 flies and among these were found four variant males, one of which was yellow scute miniature. Evidently, this male had received a yellow-scute X-chromosome in which a mutation to miniature had occurred.

Following our usual method of procedure in such cases, the male was mated to a yellow female with attached X-chromosomes. The culture gave 54 yellow females, 27 yellow scute miniature males and 22 yellow scute males. It was first supposed that it was a case of "fractional" mutation, that is, part of the germ cells of this male had received the mutated gene, and part had not. Such cases are sometimes found in X-rayed material. However, mass cultures were made both of the yellow scute miniature males and of the yellow scute males. The latter bred true to the wild type (normal wings), while the former gave 29

yellow scute miniature males and 23 yellow scute males. It was then apparent that the miniature was reverting back to the wild type, or normal wings, and that it was a case of a mutable gene, similar to the one found in *Drosophila virilis* by Demereč and extensively studied by him.

It was decided to keep stock of the new mutable gene, until an opportunity to make thorough tests could be found. From the tests already made, it seemed that it would be possible to maintain stock by selecting the miniature males in each generation and mating them in mass cultures to virgin yellow females with attached X-chromosomes. But in the next generation the mass culture gave 32 miniature males and only a single long winged male. If the mutable gene were not to be lost, it would be necessary to resort to individual matings. Accordingly ten paired and three mass culture matings were made. All of the paired matings and two of the three mass matings yielded miniature males only, but the third mass culture gave 26 miniature and nine normal winged males. In the next generation nine paired matings gave 427 males, all miniature winged, but there were obtained from a mass culture 231 miniatures and 55 normals.

The new miniature stock has been tested to normal miniature. All of the F_1 flies were miniature, showing that it is a true miniature. Another similar test was made by mating the yellow scute miniature males to virgin yellow white miniature females. The cultures gave 208 yellow miniature females and 205 yellow white miniature males. The long winged yellow scute males, derived by reversion from the miniature, continue to breed true to the wild-type (normal wings).

The mutable miniature gene in *Drosophila melanogaster* differs somewhat from that found in *D. virilis* by Demereč. Demereč has been able to obtain three distinct lines: (a) The alpha line, in which the gene is mutable both in the germ cells and in the somatic cells; (b) the gamma line, in which it is mutable in somatic cells only; (c) the beta line, in which miniature behaves as an almost constant gene.[1] So far I have been unable to find any evidence that mutations back to the wild-type occur in somatic cells. Demereč has also found three modifying genes,

[1] Demereč, M. 1929. The Proceedings of the National Academy of Sciences, Vol. 15, pp. 834–838.

which stimulate the mutability of the miniature gene. Our stock has not been tested for such possible modifiers.

Any case of a new mutable gene should be carefully tested and analyzed, for by so doing it may be possible to find a satisfactory explanation for this unique type of inheritance. The pressure of other work has not made this possible in the present case. I have therefore given all material to Mr. C. P. Oliver, who plans to make such a study.

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A CASE DEMONSTRATING THE ABSENCE OF SOMATIC INDUCTION IN DROSOPHILA

In a culture obtained from the cross of an X-rayed apricot male (w^a = apricot eye, an allelomorph of w, white eye) by a $\overline{yy}$ female ("$\overline{yy}$" indicates attached X-chromosomes homozygous for y, the gene for yellow body), all the males appeared phenotypically indistinguishable from white, and the females were $\overline{yy}$. There were 92 males and 103 females in all in this culture, and among them not a single exception to the above results was observed. In order to exclude the supposition of a possible contamination of the culture, or of the $\overline{yy}$ female taken for the cross with the X-rayed apricot male not having been virgin, this same male was crossed again with three other virgin $\overline{yy}$ females. All the 212 males obtained from these crosses also appeared to be phenotypically white, and the females $\overline{yy}$. Some of these males were again crossed with $\overline{yy}$ females, giving a second generation consisting of phenotypically white males and $\overline{yy}$ females. All this showed that in the apricot culture we had obtained a mutation phenotypically identical with the mutation "white."

Two unusual facts appeared in this connection. The first fact was that in a cross of this new mutation with homozygous white females, instead of our obtaining the expected pure line of white, that is, a line in which all males and females were white, we actually obtained white males, but all the females, contrary to expectation, appeared to be phenotypically like apricot. This showed that a transgene ("x") had arisen which was not white, but some other kind of recessive. This recessive modified the gene w^a so as to produce the white

phenotype. It lay in the X-chromosome in some other position than apricot, and lost its modifying effect when in the heterozygous state. This is why females of the composition $\frac{w^a x}{w +}$, obtained from the cross of $w^a x$ males by females homozygous for white $\left(\frac{w}{w}\right)$ produced apricot-like instead of white females $\left(\frac{w^a}{w}\right.$ females are similar in eye color to $\left.\frac{w^a}{w^a}\right)$.

A further investigation showed that the character "white" in our case was a complicated phenomenon, being produced by the joint action of two genes, the old gene apricot and a newly produced gene "ruby" (allelomorphic or identical with the previously known mutant of that name lying at locus 7.5 in the old standard map of the X-chromosome). The determination of this gene was made by the method of finding the per cent. of crossing-over between the gene for apricot and the gene in question. Among a total of 798 observed flies derived from females of the composition $\frac{w^a x}{cv\ ct\ f}$, 49 crossovers between w^a and x were obtained, or 6.1 per cent. From this we may conclude that the gene modifying the character apricot to white must lie at locus 7.8 (6.1 + 1.7, the latter being the locus of the gene apricot). This locus is very close to the previously determined locus of the known gene ruby (rb). The cross of the male carrying the new gene "x" (ruby?) with $\frac{rb}{rb}$ females, homozygous for the previously known ruby, gave a pure line of ruby. This definitely confirms the supposition of the origination, in this case, of the gene rb.

The second unusual fact is that, in the culture obtained from the cross of the male w^a by $\overline{yy}$, not only one male offspring was a mutant, as ordinarily is found to be the case at the first appearance of a mutant gene, but *all* the males of the culture at once appeared as mutants. In other words, *all* the germ cells of the X-rayed apricot male, without exception, seemed to have mutated at once, and in one direction—a case quite unthinkable from the point of view of modern genetics. Evidently the cause of this phenomenon is to be found not in simultaneous and identical mutations of all the germ cells, but in some other kind of principle. It is necessary to assume that the hereditary change in question appeared not at the time of the X-raying

of the sexually mature apricot male, whose germ cells were already differentiated, but much earlier, and precisely at that stage of its embryonic development, after the time when the cell—or rather, chromatids—destined to form the optic anlage had become separated from the germ tract, but before any cells—or rather, chromatids—of the germ tract had become separated from each other. This was probably at the first or second zygotic cell division, or at the prior chromatid division corresponding to that. In that single germ cell the new transgenation ruby must have arisen. Further development then led to the result that all germ cells had, in addition to the gene apricot, the gene ruby as well, but that the somatic cells did not have the latter. In consequence a male was obtained which carried in all his somatic cells the gene apricot, and possessed genotypically yet another additional factor—ruby.

In this way there has been obtained in Drosophila, in a natural way, a result which Castle, working on guinea pigs, Guthrie, Davenport, Shultz on chickens, Magnus on rabbits, and Kaltenbach on ducks had tried to obtain artificially. That is, we obtained a fly whose "outer case" of somatic tissue differed in its hereditary content from the germinal genotype contained within it. It is noteworthy that this "somatic case" did not exercise any detectable influence upon the germinal genotype, despite the fact that the offspring of this fly were rather numerous. The phenomenon described points to the presence of a "germinal tract" (Keimbahn) in the embryogeny of Drosophila.

Since the work of Johannsen, the ideas "phenotype" and "genotype" have been generally accepted in biology. Without these ideas modern genetics would never have reached the high level on which it is found at the present time. Nevertheless, these ideas are not always used in the same sense and at times they are given an erroneous interpretation, which leads to wrong formulations and to confusion.

Ordinarily we understand by the "phenotype" of an organism its genotype as realized under certain particular conditions. As the "genotype" we have in view a collection of genes in each germ cell. The "genotype" is understood to be the hereditary content of the organism. The term "genotypic" is considered equivalent to the term "hereditary." Very often an indissoluble unity of phenotype and genotype is implied, con-

noting a unity of the non-hereditary manifestation of a character, and the hereditary content of an organism.

It appears to us that these concepts are not quite accurate or valid, but are in need of definite correction. Above all it is necessary to point out that we can speak of the phenotype as of a realized hereditary genotype only in the sense of its connection with time, but not with space, that is, historically but not physiologically. The genotype of the germ cells of the apricot male, in the case above described, contained, in addition to the other genes, the gene ruby as well. This gene was realized in the offspring; it was not realized at all in our male, but existed in a latent state, hidden within its "somatic case." The male apricot did not realize the genotype of his own germ cells, but only that genotype of the *parents,* which he had received in his *somatic* cells. The genotype of the germ cells themselves is never realized in the organism possessing these germ cells, it manifests itself only in the offspring, and does so only insofar as it goes over into the somatic cells of these offspring. Therefore the connection between the phenotype of an organism and the hereditary genotype of its germ cells, in other words, the connection between the characters of the organism and its material carriers of heredity, is an historical connection but not at all an existing biological one. It is self-understood that this historical connection does not exclude physiological connections and reciprocal actions between the somatic and the germ cells in each given organism. But these connections have nothing in common with heredity, any more than, for instance, the physiological connections between any of the somatic organs and parts of the organism have anything in common with heredity. From the fact that we may discover a very close physiological connection between the lungs and the heart, it surely will never occur to any one to make the deduction of a *hereditary* influence of these organs on each other.

One can not consider the ideas "genotypic" and "hereditary" as identical. In each organism, as is known, there are in principle as many genotypes as there are cells, and we should in general distinguish the inherited genotype of the germ cells from the inherited genotype of the somatic cells. For, although the latter coincides with the former in an overwhelmingly great number of cases on account of the shortness of the historical path from parents to children, the two must be dis-

tinguished on principle, if only for the reason that we often have to do not only with ordinary phenotypic changes of the organism, in reaction to influences of the environment, but with definite changes in the genotype. And only in this sense can one properly speak of a unity of phenotype and genotype.

The genotype of the germ cells never manifests itself in its carrier. *It has no phenotype at all,* as long as it exists in a hidden state, as long as it does not appear. We judge the genotype of the germ cells of an observed organism only by the subsequent analysis of the phenotype of its *offspring.* The developments of the somatic and of the germ cells do not depend *genotypically* upon each other, although both come from the same root, the same fertilized egg cell, and must exist in permanent, physiological connection with one another. A genotypic change of the "root" leads to a change in both branches growing out of it; a change, however, in some part of one of these branches is not reflected correspondingly in the other.

All these facts show once more how far modern biology has gone beyond the reasonings of the Lamarckians concerning so-called "somatic induction," beyond their naïve faith in the inheritance of acquired characters.

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CHANGE IN COLOR PATTERN IN A CAPTIVE RED SQUIRREL

AN immature male red squirrel (*Sciurus hudsonicus loquax*), one of a litter of five born in captivity on August 20, 1927, showed a marked deviation from the normal coloration of this species. The mother of this litter was captured alive and uninjured at Holland, Michigan, on August 5, 1927.

The unusual coloration consisted of a number of light gray spots on the dark gray and reddish-brown body. The locations of the spots were as follows: on the neck, a small area back of each ear; a large triangular patch on the back, extending from the shoulders to a point half-way down the back of the animal; a circular spot on each side of the body, midway between the belly and the dorsal line, just anterior to the hind legs, and one on each side of the rump at the base of the tail, the latter being totally gray (Fig. 1).

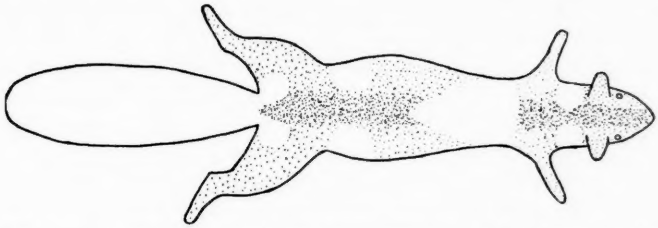

FIG. 1. Illustrating the maximum development of the light gray patches on April 4, 1928.

The grayness of the spots was found to be due to the presence of a large number of unpigmented hairs among the normally colored ones, the combination of the two giving the light gray appearance noted. This abnormality was first noticed during the spring of 1928 when the squirrel was eight months old.

At this time of the year, the other squirrels of the same litter, as well as wild squirrels in the immediate vicinity, were still in the dark red winter pelage. The odd coloration of this particular animal contrasted strongly with the normal red pelage.

As soon as discovered, this squirrel was separated from his cage-mates and observed. Drawings were made of the animal on April 4, when first discovered and separated, on April 13 and on May 6, 1928, in order to show the changes occurring in the pelage.

Previous to its isolation, this squirrel was very seldom seen, as it persisted in remaining in the nest box. When it did come out to feed, it was tormented and chased by its cage-mates until forced to seek refuge in the nest box. For this reason, the abnormal coloration was not discovered sooner. Soon after its separation, the squirrel lost most of its wildness and became more active, coming out of the nest box to feed during the daytime and to run a wheel placed in its cage.

Nine days after isolation the gray patches began to disappear. The two side patches in front of the hind legs and those at the base of the tail had entirely disappeared. The large dorsal patch had broken up into two small areas on the sides of the body and were still in the process of disappearance as they were not as light a gray as the area still remaining on the neck nor as dark a gray as the rest of the body. The two spots on the neck had fused into one narrow band extending clear across the base of the neck. The anterior half of the tail became more deeply pig-

mented and as dark a gray as the rest of the body, while the tip half still remained light gray. The disappearance of the patches was evidently accomplished by the dropping out of the unpigmented hairs and the growth of the normally colored ones.

On May 6, or thirty-two days after separation, all the light gray patches had disappeared except that on the tip of the tail, which still remained as when first discovered. When I had left for the summer, during the first part of June, this tip was still light gray. I am unable to state how long this grayness persisted, as the squirrel escaped during my absence, curtailing all further observations.

G. M. Allen,[1] in 1914, offered a theory to account for the color patterns in mammals and birds. Allen's theory may be recapitulated as follows:

Pigment production is more intense at certain definite centers on the body, and the occurrence of white or pigmentless areas is due to the restriction of pigment formation at the periphery of these centers, so that white occurs at their extremities or as breaks between contiguous color patches. There are eleven of these areas or centers on the body: one median coronal or crown patch, two aural or ear patches, two neck or nuchal patches, two scapular or shoulder patches, two side or pleural centers and two sacral or rump areas. When an animal is completely pigmented, all the centers are fully developed. When it is a total albino, none of the pigment is produced. Between these two extremes come the various "piebalds" or particolored animals that are common among domestic as well as wild animals. Allen, in his paper, gives many illustrations of this phenomenon.

This theory applies very well to my observations on the red squirrel. When the specimen was first discovered, the crown patch was well developed. The ear centers were but partially developed as they were still only a light gray. The neck areas were also not fully developed as they were also light gray. In the scapular region, the pigment production was only partially complete, for a large light gray patch separated this area from the side centers of pigmentation. Two light gray patches in the posterior portion of the body showed that the sacral center was not well developed. A patch of light gray separated the pleural from the sacral areas anteriorly, and a large posterior gray area

[1] Allen, G. M., "Pattern Development in Mammals and Birds," AMER. NAT., Vol. 48, 1914, pp. 385–415, 467–484, 550–556.

suggested that the sacral pigmentation was restricted so that the entire tail region was not pigmented.

It seems that when the squirrel was given plenty of food and rest, the physiological processes improved. The squirrel tended to become more normal in action. That coloration also became more normal is evidenced by the reduction in the size and final disappearance of the light gray areas.

On May 6 and until June 2, all the light gray patches had disappeared except that on the tip of the tail, which still remained only partially pigmented. As white-tailed red squirrels are known to occur in nature (as recorded by Allen in the above-mentioned paper), it may be probable that either the sacral pigment center is the least developed of all the pigment production centers or that pigment production is first restricted in this region.

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A THUMB TEST FOR DISTINGUISHING MAZZARD (*PRUNUS AVIUM* L.) AND MAHALEB (*PRUNUS MAHALEB* L.) ROOTSTOCKS

INVESTIGATIONS upon cherry orchards frequently call for the need of ascertaining whether *Mazzard* or *Mahaleb* furnish the roots of the respective trees. Well-trained nurserymen and horticulturists can quite easily distinguish the two stocks by their morphological characters. Nevertheless, a check upon these observations may be of aid in final decisions.

Small pieces of roots of approximately the same size from *Mazzard* and *Mahaleb* cherries are immersed in separate vials containing an aqueous solution of from 4 to 10 per cent. iron alum. In a few minutes the solution containing the *Mazzard* roots commences to darken. This darkening reaches its fullest intensity in from one to forty-eight hours according to the size of the pieces and the amount of liquid employed. The vial containing the *Mahaleb* roots remains practically clear and undarkened. The test has worked satisfactorily so far under all conditions of application. Roots of trees of different size and vigor have been tested at all times of the year from May

to November. The test has not been made during the winter months, as May, 1930, represents the date of its initiation.

The differential change in color is doubtlessly due to the differential quantitative presence of soluble tannic substances. From Wehmer (1), page 478, I may quote: "P. avium L.—Rinde n. aelteren Angaben: Gallussaeure, Tannin, neuerdings—Phlobaphen, Eisengruen Tannin—." For *P. mahaleb* no tannic substances are listed. (This is not taken to mean that *Mahaleb* is entirely devoid of tannic substances).

Czapek (2), p. 499, Vol. iii, and Abderhalden (3), page 1, Vol. 7, use "tannic substances" as a circumscriptive term. I wish it to be understood in the same way. It is therefore thought that the test can be carried out with any of the various reagents for detecting tannic substances listed in both these textbooks on biochemistry. Some of the methods have already been successfully tested by Mr. H. B. Tukey. The author wishes to express his thanks to Messrs. H. B. Tukey and K. Brase for giving the method numerous field trials.

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